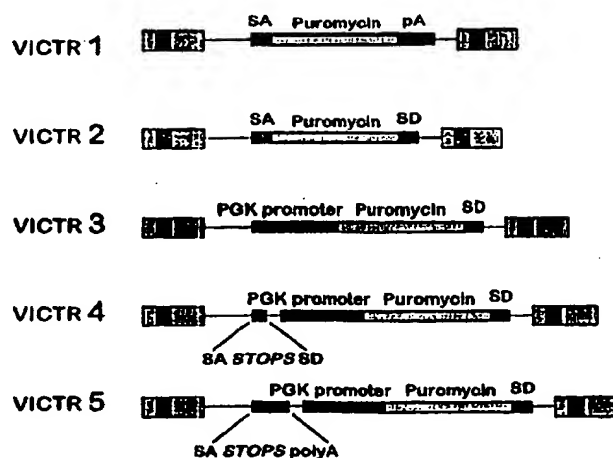




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(54) Title: AN INDEXED LIBRARY OF CELLS CONTAINING GENOMIC MODIFICATIONS AND METHODS OF MAKING AND UTILIZING THE SAME



(57) Abstract

Methods and vectors (both DNA and retroviral) are provided for the construction of a Library of mutated cells. The Library will preferably contain mutations in essentially all genes present in the genome of the cells. The nature of the Library and the vectors allow for methods of screening for mutations in specific genes, and for gathering nucleotide sequence data from each mutated gene to provide a database of tagged gene sequences. Such a database provides a means to access the individual mutant cell lines contained in the Library. The invention includes the described Library, methods of making the same, and vectors used to construct the Library. Methods are also provided for accessing individual parts of the Library either by sequence or by pooling and screening. The invention also provides for the generation of non-human transgenic animals which are mutant for specific genes as isolated and generated from the cells of the Library.

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AN INDEXED LIBRARY OF CELLS CONTAINING GENOMIC MODIFICATIONS
AND METHODS OF MAKING AND UTILIZING THE SAME

The present application claims priority to U.S.
5 Applications Ser. Nos. 08/726,867, filed October 4, 1996,
08/728,963, filed October 11, 1996, and 08/907,598, filed
August 8, 1997, the disclosures of which are herein
incorporated by reference.

10 1.0. FIELD OF THE INVENTION

The invention relates to an indexed library of
genetically altered cells and methods of organizing the cells
into an easily manipulated and characterized Library. The
invention also relates to methods of making the library,
15 vectors for making insertion mutations in genes, methods of
gathering sequence information from each member clone of the
Library, and methods of isolating a particular clone of
interest from the Library.

20 2.0. BACKGROUND OF THE INVENTION

The general technologies of targeting mutations into the
genome of cells, and the process of generating mouse lines
from genetically altered embryonic stem (ES) cells with
specific genetic lesions are well known (Bradley, 1991, Cur.
25 Opin. Biotech. 2:823-829). A random method of generating
genetic lesions in cells (called gene, or promoter, trapping)
has been developed in parallel with the targeted methods of
genetic mutation (Allen et al., 1988 Nature 333(6176):852-
855; Brenner et al., 1989, Proc. Natl. Acad. Sci. U.S.A.
30 86(14):5517-5521; Chang et al., 1993, Virology 193(2):737-
747; Friedrich and Soriano, 1993, Insertional mutagenesis by
retroviruses and promoter traps in embryonic stem cells, p.
681-701. In Methods Enzymol., vol. 225., P. M. Wassarman and
M. L. DePamphilis (ed.), Academic Press, Inc., San Diego;
35 Friedrich and Soriano, 1991, Genes Dev. 5(9):1513-1523;
Gossler et al., 1989, Science 244(4903):463-465; Kerr et al.,
1989, Cold Spring Harb. Symp. Quant. Biol. 2:767-776; Reddy
et al., 1991, J Virol. 65(3):1507-1515; Reddy et al., 1992,

Proc. Natl. Acad. Sci. U.S.A. 89(15):6721-6725; Skarnes et al., 1992, Genes Dev. 6(6):903-918; von Melchner and Ruley, 1989, J. Virol. 63(8):3227-3233; Yoshida et al., 1995, Transgen. Res. 4:277-287). Gene trapping provides a means to
5 create a collection of random mutations by inserting fragments of DNA into transcribed genes. Insertions into transcribed genes are selected over the background of total insertions since the mutagenic DNA encodes an antibiotic resistance gene or some other selectable marker. The
10 selectable marker lacks its own promoter and enhancer and must be expressed by the endogenous sequences that flank the marker after it has integrated. Using this approach, transcription of the selectable marker is activated and the cell gene is concurrently mutated. This type of strict
15 selection makes it possible to easily isolate thousands of ES cell colonies, each with a unique mutagenic insertion.

Collecting mutants on a large-scale has been a powerful genetic technique commonly used for organisms which are more amenable to such analysis than mammals. These organisms,
20 such as *Drosophila melanogaster*, yeast *Saccharomyces cerevisiae*, and plants such as *Arabidopsis thaliana* are small, have short generation times and small genomes (Bellen et al., 1989, Genes Dev. 3(9):1288-1300; Bier et al., 1989, Genes Dev. 3(9):1273-1287; Hope, 1991, Develop. 113(2):399-408.
25 These features allow an investigator to rear many thousands or millions of different mutant strains without requiring unmanageable resources. However, these type of organisms have only limited value in the study of biology relevant to human physiology and health. It is therefore important to
30 have the power of large-scale genetic analysis available for the study of a mammalian species that can aid in the study of human disease. Given that the entire human genome is presently being sequenced, the comprehensive genetic analysis of a related mammalian species will provide a means to
35 determine the function of genes cloned from the human genome. At present, rodents, and particularly mice, provide the best model for genetic manipulation and analysis of mammalian

physiology.

Gene trapping has been used as an analytical tool to identify genes and regulatory regions in a variety of animal cell types. One system that has proved particularly useful
5 is based on the use of ROSA (reverse orientation splice acceptor) retroviral vectors (Friedrich and Soriano, 1991 and 1993).

The ROSA system can generate mutations that result in a detectable homozygous phenotype with a high frequency. About
10 50% of all the insertions caused embryonic lethality. The specifically mutated genes may easily be cloned since the gene trapping event produces a fusion transcript. This fusion transcript has trapped exon sequences appended to the sequences of the selectable marker allowing the latter to be
15 used as a tag in polymerase chain reaction (PCR)-based protocols, or by simple cDNA cloning. Examples of genes isolated by these methods include a transcription factor related to human TEF-1 (transcription enhancer factor-1) which is required in the development of the heart (Chen et
20 al., 1994, Genes Devel. 8:2293-2301. Another (spock), is distantly related to yeast genes encoding secretion proteins and is important during gastrulation.

The above experiments have established that the ROSA system is an effective analytical tool for genetic analysis
25 in mammals. However, the structure of many ROSA vectors selects for the "trapping" of 5' exons which, in many cases, do not encode proteins. Such a result is adequate where one wishes to identify and eventually clone control (i.e., promoter or enhancer) sequences, but is not optimal where the
30 generation of insertion-inactivated null mutations is desired, and relevant coding sequence is needed. Thus, the construction of large-scale mutant (preferably null mutant) libraries requires the use of vectors that have been designed to select for insertion events that have occurred within the
35 coding region of the mutated genes as well as vectors that are not limited to detecting insertions into expressed genes.

3.0. SUMMARY OF THE INVENTION

An object of the present invention is to provide a set of genetically altered cells (the 'Library'). The genetic alterations are of sufficient randomness and frequency such
5 that the combined population of cells in the Library represent mutations in essentially every gene found in the cell's genome. The Library is used as a source for obtaining specifically mutated cells, cell lines derived from the individually mutated cells, and cells for use in the
10 production of transgenic non-human animals.

A further object is to provide the vectors, both DNA and retroviral based, that may be used to generate the Library. Typically, at least two distinct vector designs will be used in order to mutate genes that are actively expressed in the
15 target cell, and genes that are not expressed in the target cell. Combining the mutant cells obtained using both types of vectors best ensures that the Library provides a comprehensive set of gene mutations.

A particularly useful vector class contemplated by the
20 present invention includes a vector for inserting foreign exons into animal cell transcripts that comprises a selectable marker, a promoter element operatively positioned 5' to the selectable marker, a splice donor site operatively positioned 3' to the selectable marker, and a second
25 mutagenic foreign polynucleotide sequence located upstream from the promoter element that disrupts, or otherwise "poisons", the splicing or read-through expression of the endogenous cellular transcript. Typically, the mutagenic foreign polynucleotide sequence may incorporate a
30 polyadenylation (pA) site, a nested set of stop codons in each of the three reading frames, splice acceptor and splice donor sequences in operable combination, a mutagenic exon, or any mixture of mutagenic features that effectively prevent the expression of the cellular gene. For example, a
35 polyadenylation sequence may be incorporated in addition to or in lieu of the splice donor sequence. A preferred organization for the mutagenic polynucleotide sequence

comprises a polyadenylation site positioned upstream from a selectable marker which is in turn located upstream from a splice acceptor sequence. Preferably, such a vector does not comprise a transcription terminator or polyadenylation site 5 operatively positioned relative to the coding region of the selectable marker, and shall not comprise a splice acceptor site operatively positioned between the promoter element and the initiation codon of said selectable marker.

An additional vector contemplated by the present 10 invention is designed to replace the normal 3' end of an animal cell transcript with a foreign exon. Such a vector shall generally be engineered to comprise a selectable marker, a splice acceptor site operatively positioned upstream (5') from the initiation codon of the selectable 15 marker, and a polyadenylation site operatively positioned downstream (3') from the termination codon (3' end) of the selectable marker. Preferably, the vector will not comprise a promoter element operatively positioned upstream from the coding region of the selectable marker, and will not comprise 20 a splice donor sequence operatively positioned between the 3' end of the coding region of the selectable marker and the polyadenylation site.

Yet another vector contemplated by the present invention is a vector designed to insert a mutagenic foreign 25 polynucleotide sequence within an animal cell transcript (i.e., the foreign polynucleotide sequence is flanked on both sides by endogenous exons). As described above, the mutagenic foreign polynucleotide sequence may be any sequence that disrupts the normal expression of the gene into which 30 the vector has integrated. Optionally, the vector may additionally incorporate a selectable marker, a splice acceptor site operatively positioned 5' to the initiation codon of the selectable marker, a splice donor site operatively positioned 3' to said selectable marker. 35 Preferably, this vector shall not comprise a polyadenylation site operatively positioned 3' to the coding region of said selectable marker, and shall not comprise a promoter element

operatively positioned 5' to the coding region of said selectable marker.

An additional embodiment of the present invention is a library of genetically altered cells that have been treated to stably incorporate one or more types of the vectors described above. The presently described library of cultured animal cells may be made by a process comprising the steps of treating (i.e., infecting, transfecting, retrotransposing, or virtually any other method of introducing polynucleotides into a cell) a population of cells to stably integrate a vector that mediates the splicing of a foreign exon internal to a cellular transcript, transfecting another population of cells to stably integrate a vector that mediates the splicing of a foreign exon 5' to an exon of a cellular transcript, and selecting for transduced cells that express the products encoded by the foreign exons.

Alternatively, an additional embodiment of the present invention describes a mammalian cell library made by a method comprising the steps of: transfecting a population of cells with a vector capable of expressing a selectable marker in the cell only after the vector inserts into the host genome; transfecting or infecting a population of cells with a vector containing a selectable marker that is substantially only expressed by cellular control sequences (after the vector integrates into the host cells genome); and growing the transfected cells under conditions that select for the expression of the selectable marker.

In an additional embodiment of the present invention, the two populations of transfected cells will be individually grown under selective conditions, and the resulting mutated population of cells collectively comprises a substantially comprehensive library of mutated cells.

In an additional embodiment of the present invention, the individual mutant cells in the library are separated and clonally expanded. Additionally, the clonally expanded mutant cells may then be analyzed to ascertain the DNA

sequence, or partial DNA sequence of the mutated host gene.

The presently described methods of making, organizing, and indexing libraries of mutated animal cells are also broadly applicable to virtually any eukaryotic cells that may
5 be genetically manipulated and grown in culture.

The invention provides for sequencing every gene mutated in the Library. The resulting sequence database subsequently serves as an index for the library. In essence, every cell line in the Library is individually catalogued using the
10 partial sequence information. The resulting sequence is specific for the mutated gene since the present methods are designed to obtain sequence information from exons that have been spliced to the marker sequence. Since the coverage of the mutagenesis is preferably the entire set of genes in the
15 genome, the resulting Library sequence database contains sequence from essentially every gene in the cell. From this database, a gene of interest can be identified. Once identified, the corresponding mutant cell may be withdrawn from the Library based on cross reference to the sequence
20 data.

An additional embodiment of the invention provides for methods of isolating mutations of interest from the Library. Two methods are proposed for obtaining individual mutant cell lines from the Library. The first provides a scheme where
25 clones of the cells generated using the above vectors are pooled into sets of defined size. Using the procedure described below which utilizes reverse transcription (RT) and polymerase chain reaction (PCR), a cell line with a mutation in a gene whose sequence is partly or wholly known is
30 isolated from organized sets of these pools. A few rounds of this screening procedure results in the isolation of the desired individual cell line.

A second procedure involves the sequencing of regions flanking the vector insertion sites in the various cells in
35 the library. The sequence database generated from these data effectively constitutes an index of the clones in the library that may be used to identify cells having mutations in

specific genes.

4.0. DESCRIPTION OF THE FIGURES

Figure 1. Shows a diagrammatic representation of 5 different vectors that are generally representative of the type of vectors that may be used in the present invention.

Figure 2. Shows a general strategy for identifying "trapped" cellular sequences by PCR analysis of the cellular exons that flank the foreign intron introduced by the VICTR 2 vector.

Figure 3 shows a PCR based strategy for identifying tagged genes by chromosomal location.

Figure 4. Is a diagrammatic representation of a strategy of identifying or indexing the specific clones in the library via PCR analysis and sequencing of mRNA samples obtained from the cells in the library.

Figure 5. Is a diagrammatic representation of a method of isolating positive clones by screening pooled mutant cell clones.

Figure 6. Partial nucleic acid or predicted amino acid sequence data from 9 clones (OST1-9) isolated using the described techniques aligned with similar sequences from previously characterized genes.

Figure 7. Provides a diagrammatic representation of VICTRs and 20 as well as the transcripts that result after integration into a hypothetical region of the target cell genome (i.e., "Wildtype Locus").

Figure 8. Provides a representative list of a portion of the known genes that have been identified using the disclosed methods and technology.

5.0. DETAILED DESCRIPTION OF THE INVENTION

The present invention describes a novel indexed library containing a substantially comprehensive set of mutations in the host cell genome, and methods of making and using the same. The presently described Library comprises as a set of cell clones that each possess at least one mutation (and preferably a single mutation) caused by the insertion of DNA that is foreign to the cell. For the purposes of the present invention, "foreign" polynucleotide sequences can be any sequences that are newly introduced to a cell, do not naturally occur in the cell at the engineered region of the chromosome, or occur in the cell but are not organized to provide an identical function to that provided in the engineered vector.

The particularly novel features of the Library include the methods of construction, and indexing. To index the library, the mutant cells of the library are clonally expanded and each mutated gene is at least partially sequenced. The Library thus provides a novel tool for assessing the specific function of a given gene. The insertions cause a mutation which allow for essentially every gene represented in the Library to be studied using genetic techniques either *in vitro* or *in vivo* (via the generation of transgenic animals). For the purposes of the present invention, the term "essentially every gene" shall refer to the statistical situation where there is generally at least about a 70 percent probability that the genomes of cells used to construct the library collectively contain at least one inserted vector sequence in each gene, preferably a 85 percent probability, and more specifically at least about a 95 percent probability as determined by a standard Poisson distribution.

Also for the purposes of the present invention the term "gene" shall refer to any and all discrete coding regions of the cell's genome, as well as associated noncoding and regulatory regions. Additionally, the term operatively positioned shall refer to the control elements or genes that

are provided with the proper orientation and spacing to provide the desired or indicated functions of the control elements or genes.

For the purposes of the present invention, a gene is
5 "expressed" when a control element in the cell mediates the production of functional or detectable levels of mRNA encoded by the gene, or a selectable marker inserted therein. A gene is not expressed where the control element in the cell is absent, has been inactivated, or does not mediate the
10 production of functional or detectable levels of mRNA encoded by the gene, or a selectable marker inserted therein.

5.1. Vectors used to build the Library

A number of investigators have developed gene trapping
15 vectors and procedures for use in mouse and other cells (Allen et al., 1988; Bellen et al., 1989, Genes Dev. 3(9):1288-1300; Bier et al., 1989, Genes Dev. 3(9):1273-1287; Bonnerot et al., 1992, J Virol. 66(8):4982-4991; Brenner et al., 1989; Chang et al., 1993; Friedrich and Soriano, 1993;
20 Friedrich and Soriano, 1991; Goff, 1987, Methods Enzymol. 152:469-481; Gossler et al.; Hope, 1991; Kerr et al., 1989; Reddy et al., 1991; Reddy et al., 1992; Skarnes et al., 1992; von Melchner and Ruley; Yoshida et al., 1995). The gene trapping system described in the present invention is based
25 on significant improvements to the published SA (splice acceptor) DNA vectors and the ROSA (reverse orientation, splice acceptor) retroviral vectors (Chen et al., 1994; Friedrich and Soriano, 1991 and 1993). The presently described vectors also use a selectable marker called β geo.
30 This gene encodes a protein which is a fusion between the β -galactosidase and neomycin phosphotransferase proteins. The presently described vectors place a splice acceptor sequence upstream from the β geo gene and a poly-adenylation signal sequence downstream from the marker. The marker is
35 integrated after transfection by, for example, electroporation (DNA vectors), or retroviral infection, and gene trap events are selected based on resistance to G418

resulting from activation of β geo expression by splicing from the endogenous gene into the ROSA splice acceptor. This type of integration disrupts the transcription unit and preferably results in a null mutation at the locus.

5 Although gene trapping has proven a useful analytical tool, the present invention contemplates gene trapping on a large scale. The vectors utilized in the present invention have been engineered to overcome the shortcomings of the early gene trap vector designs, and to facilitate procedures
10 allowing high throughput. In addition, procedures are described that allow the rapid and facile acquisition of sequence information from each trapped cDNA which may be adapted to allow complete automation. These latter procedures are also designed for flexibility so that
15 additional molecular information can easily be obtained subsequently. The present invention therefore incorporates gene trapping into a larger and unique tool. A specially organized set of gene trap clones that provide a novel and powerful new tool of genetic analysis.

20 The presently described vectors are superficially similar to the ROSA family of vectors, but constitute significant improvements and provide for additional features that are useful in the construction and indexing of the Library. Typically, gene trapping vectors are designed to
25 detect insertions into transcribed gene regions within the genome. They generally consist of a selectable marker whose normal expression is handicapped by exclusion of some element required for proper transcription. When the vector integrates into the genome, and acquires the necessary
30 element by juxtaposition, expression of the selectable marker is activated. When such activation occurs, the cell can survive when grown in the appropriate selective medium which allows for the subsequent isolation and characterization of the trapped gene. Integration of the gene trap generally
35 causes the gene at the site of integration to be mutated.

Some gene trapping vectors have a splice acceptor preceding a selectable marker and a poly-adenylation signal

following the selectable marker, and the selectable marker gene has its own initiator ATG codon. Using this arrangement, the fusion transcripts produced after integration generally only comprise exons 5' to the insertion site to the known marker sequences. Where the vector has been inserted into the 5' region of the gene, it is often the case that the only exon 5' to the vector is a non-coding exon. Accordingly, the sequences obtained from such fusions do not provide the desired sequence information about the relevant gene products. This is because untranslated sequences are generally less well conserved than coding sequences.

To compensate for the short-comings of earlier vectors, the vectors of the present invention have been designed so that 3' exons are appended to the fusion transcript by replacing the poly-adenylation and transcription termination signals of earlier ROSA vectors with a splice donor (SD) sequence. Consequently transcription and splicing generally results in a fusion between all or most of the endogenous transcript and the selectable marker exon, for example β geo, neomycin (neo) or puromycin (puro). The exon sequences immediately 3' to the selectable marker exon may then be sequenced and used to establish a database of expressed sequence tags. The presently described procedures will typically provide approximately 200 nucleotides of sequence, or more. These sequences will generally be coding and therefore informative. The prediction that the sequence obtained will be from coding region is based on two factors. First, gene trap vectors are generally found near the 5' end of the gene immediately after untranslated exons because the method selects for integration events that place the initiator ATG of the selectable marker as the first encountered, and thus used, for translation. Second, mammalian transcripts have short 5' untranslated regions (UTRs) which are typically between 50 and 150 nucleotides in length.

The obtained sequence information also provides a ready source of probes that may be used to isolate the full-length

gene or cDNA from the host cell, or as heterologous probes for the isolation of homologous genes in other species.

Internal exons in mammalian transcripts are generally quite small, on the average 137 bases with few over 300
5 bases. Consequently, a large internal exon may be spliced less efficiently. Thus, the presently described vectors have been designed to sandwich relatively small selectable markers (for example: neo, ~800 bases, or a smaller drug resistance gene such as puro, ~600 bases) between the requisite splicing
10 elements to produce relatively small exons. Exons of this size are more typical of mammalian exons and do not present undue problems for the splicing machinery of the cell. Such a design consideration is novel to the presently disclosed gene trapping vectors. Accordingly, an additional embodiment
15 of the claimed vectors is that the respective splice acceptor and splice donor sites are engineered such that they are operatively positioned close to the ends of the selectable marker coding region (the region spanning from the initiation codon to the termination codon). Generally, the splice
20 acceptor or splice donor sequences shall appear within about 80 bases from the nearest end of the selectable marker coding region, preferably within about 50 bases from the nearest end of the coding region, more preferably within about 30 bases from the nearest end of the coding regions and specifically
25 within about 20 bases of the nearest end of the selectable marker coding region.

The new vectors are represented in retroviral form in Figure 1. They are used by infecting target cells with retroviral particles such that the proviruses shown in the
30 schematic can be found in the genome of the target. These vectors are called VICTR which is an acronym for "viral constructs for trapping".

The presently described retroviral vectors may be used in conjunction with retroviral packaging cell lines such as
35 those described in U.S. Patent No. 5,449,614 ("'614 patent") issued September 12, 1995, herein incorporated by reference. Where non-mouse animal cells are to be used as targets for

generating the described libraries, packaging cells producing retrovirus with amphotropic envelopes will generally be employed to allow infection of the host cells.

The mutagenic gene trap DNA may also be introduced into
5 the target cell genome by various transfection techniques which are familiar to those skilled in the art such as electroporation, lipofection, calcium phosphate precipitation, infection, retrotransposition, and the like. Examples of such techniques may be found in Sambrook et al.
10 (1989) Molecular Cloning Vols. I-III, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, and Current Protocols in Molecular Biology (1989) John Wiley & Sons, all Vols. and periodic updates thereof, herein incorporated by reference. The transfected versions of the retroviral
15 vectors are typically plasmid DNA molecules containing DNA cassettes comprising the described features between the retroviral LTRs.

The vectors VICTR 1 and 2 (Fig. 1) are designed to trap genes that are transcribed in the target cell. To trap genes
20 that are not expressed in the target cell, gene trap vectors such as VICTR 3, 4 and 5 (described below) are provided. These vectors have been engineered to contain a promoter element capable of initiating transcription in virtually any cell type which is used to transcribe the coding sequence of
25 the selectable marker. However, in order to get proper translation of the marker product, and thus render the cell resistant to the selective antibiotic, a polyadenylation signal and a transcription termination sequence must be provided. Vectors VICTR 3 through 5 are constructed such
30 that an effective polyadenylation signal can only be provided by splicing with an externally provided downstream exon that contains a poly-adenylation site. Therefore, since the selectable marker coding region ends only in a splice donor sequence, these vectors must be integrated into a gene in
35 order to be properly expressed. In essence, these vectors append the foreign exon encoding the marker to the 5' end of an endogenous transcript. These events will tag genes and

create mutations that are used to make clones that will become part of the Library.

With the above design considerations, the VICTR series of vectors, or similarly designed and constructed vectors, have the following features. VICTR 1 is a terminal exon gene trap. VICTR 1 does not contain a control region that effectively mediates the expression of the selectable marker gene. Instead, the coding region of the selectable marker contained in VICTR 1, in this case encoding puromycin resistance (but which can be any selectable marker functional in the target cell type), is preceded by a splice acceptor sequence and followed by a polyadenylation addition signal sequence. The coding region of the puro gene has an initiator ATG which is downstream and adjacent to a region of sequence that is most favorable for translation initiation in eukaryotic cells - the so called Kozak consensus sequence (Kozak, 1989, J. Cell, Biol. 108(2):229-241). With a Kozak sequence and an initiator ATG, the puro gene in VICTR 1 is activated by integrating into the intron of an active gene, and the resulting fusion transcript is translated beginning at the puromycin initiation (ATG/AUG) codon. However, terminal gene trap vectors need not incorporate an initiator ATG codon. In such cases, the gene trap event requires splicing and the translation of a fusion protein that is functional for the selectable marker activity. The inserted puromycin coding sequence must therefore be translated in the same frame as the "trapped" gene.

The splice acceptor sequence used in VICTR 1 and other members of the VICTR series is derived from the adenovirus major late transcript splice site located at the intron 1/exon 2 boundary. This sequence contains a polypyrimidine stretch preceding the AG dinucleotide which denotes the actual splice site. The presently described vectors contemplate the use of any similarly derived splice acceptor sequence. Preferably, the splice acceptor site will only rarely, if ever, be involved in alternative splicing events.

The polyadenylation signal at the end of the puro gene is derived from the bovine growth hormone gene. Any similarly derived polyadenylation signal sequence could be used if it contains the canonical AATAAA and can be demonstrated to terminate transcription and cause a polyadenylate tail to be added to the engineered coding exons.

VICTR 2 is a modification of VICTR 1 in which the polyadenylation signal sequence is removed and replaced by a splice donor sequence. Like VICTR 1, VICTR 2 does not contain a control region that effectively mediates the expression of the selectable marker gene. Typically, the splice donor sequence to be employed in a VICTR series vector shall be determined by reference to established literature or by experimentation to identify which sequences properly initiate splicing at the 5' end of introns in the desired target cell. The specifically exemplified sequence, AGGTAAGT, results in splicing occurring in between the two G bases. Genes trapped by VICTR 2 splice upstream exons onto the puro exon and downstream exons onto the end of the puro exon. Accordingly, VICTR 2 effectively mutates gene expression by inserting a foreign exon in-between two naturally occurring exons in a given transcript. Again, the puro gene may or may not contain a consensus Kozak translation initiation sequence and properly positioned ATG initiation codon. As discussed above, gene trapping by VICTR 1 and VICTR 2 requires that the mutated gene is expressed in the target cell line. By incorporating a splice donor into the VICTR traps, transcript sequences downstream from the gene trap insertion can be determined. As described above, these sequences are generally more informative about the gene mutated since they are more likely to be coding sequences. This sequence information is gathered according to the procedures described below.

VICTR 3, VICTR 4 and VICTR 5 are gene trap vectors that do not require the cellular expression of the endogenous trapped gene. The VICTR vectors 3 through 5 all comprise a

promoter element that ensures that transcription of the selectable marker would be found in all cells that have taken up the gene trap DNA. This transcription initiates from a promoter, in this case the promoter element from the mouse phosphoglycerate kinase (PGK) gene. However, since the constructs lack a polyadenylation signal there can be no proper processing of the transcript and therefore no translation. The only means to translate the selectable marker and get a resistant cell clone is by acquiring a polyadenylation signal. Since polyadenylation is known to be concomitant with splicing, a splice donor is provided at the end of the selectable marker. Therefore, the only positive gene trap events using VICTR 3 through 5 will be those that integrate into a gene's intron such that the marker exon is spliced to downstream exons that are properly polyadenylated. Thus genes mutated with the VICTR vectors 3 through 5 need not be expressed in the target cell, and these gene trap vectors can mutate all genes having at least one intron. The design of VICTR vectors 3 through 5 requires a promoter element that will be active in the target cell type, a selectable marker and a splice donor sequence. Although a specific promoter was used in the specific embodiments, it should be understood that appropriate promoters may be selected that are known to be active in a given cell type. Typically, the considerations for selecting the splice donor sequence are identical to those discussed for VICTR 2, *supra*.

VICTR 4 differs from VICTR 3 only by the addition of a small exon upstream from the promoter element of VICTR 4. This exon is intended to stop normal splicing of the mutated gene. It is possible that insertion of VICTR 3 into an intron might not be mutagenic if the gene can still splice between exons, bypassing the gene trap insertion. The exon in VICTR 4 is constructed from the adenovirus splice acceptor described above and the synthetic splice donor also described above. Stop codons are placed in all three reading frames in the exon, which is about 100 bases long. The stops would truncate the endogenous protein and presumably cause a

mutation.

A conceptually similar alternative design uses a terminal exon like that engineered into VICTR 5. Instead of a splice donor, a polyadenylation site is used to terminate transcription and produce a truncated message. Stops in all three frames are also provided to truncate the endogenous protein as well as the resulting transcript.

VICTR 20 is a modified version of VICTR 3 that incorporates a polyadenylation site 5' to the PGK promoter, the IRES β geo sequence (i.e., foreign mutagenic polynucleotide sequence) 5' to the polyadenylation site, and a splice acceptor site 5' to the IRES β geo coding region. VICTR 20 additionally incorporates, in operable combination, a pair of recombinase recognition sites that flank the PGKpuroSD cassette.

All of the traps of the VICTR series are designed such that a fusion transcript is formed with the trapped gene. For all but VICTR 1, the fusion contains cellular exons that are located 3' to the gene trap insertion. All of the flanking exons may be sequenced according to the methods described in the following section. To facilitate sequencing, specific sequences are engineered onto the ends of the selectable marker (e.g., puromycin coding region). Examples of such sequences include, but are not limited to unique sequences for priming PCR, and sequences complementary to the standard M13 forward sequencing primer. Additionally, stop codons are added in all three reading frames to ensure that no anomalous fusion proteins are produced. All of the unique 3' primer sequences are followed immediately by the synthetic 9 base pair splice donor sequence. This keeps the size of the exon comprising the selectable marker (puro gene) at a minimum to best ensure proper splicing, and positions the amplification and sequencing primers immediately adjacent to the flanking "trapped" exons to be sequenced as part of the construction of a Library database.

When any members of the VICTR series are constructed as retroviruses, the direction of transcription of the

selectable marker is opposite to that of the direction of the normal transcription of the retrovirus. The reason for this organization is that the transcription elements such as the polyadenylation signal, the splice sites and the promoter elements found in the various members of the VICTR series interfere with the proper transcription of the retroviral genome in the packaging cell line. This would eliminate or significantly reduce retroviral titers. The LTRs used in the construction of the packaging cell line are self-inactivating. That is, the enhancer element is removed from the 3' U3 sequences such that the proviruses resulting from infection would not have an enhancer in either LTR. An enhancer in the provirus may otherwise affect transcription of the mutated gene or nearby genes.

Since a 'cryptic' splice donor sequence is found in the inverted LTRs, this splice donor sequence has been removed from the VICTR vectors by site specific mutagenesis. It was deemed necessary to remove this splice donor so that it would not affect the trapping splicing events.

The present disclosure also describes vectors that incorporate a new way to conduct positive selection. VICTR 3 and VICTR 20 are two examples of such vectors. Both VICTR 3 and VICTR 20, contain PGKpuroSD which must splice into exons of gene that provide a polyadenylation addition sequence in order to allow expression of the puromycin selectable marker gene. When placed in a targeting vector, PGKpuroSD allows for positive selection when targeting takes place. In addition to providing positive selection, targeted events among resistant colonies are easy to identify by the 3' RACE protocols (see section 5.2.2., *infra*) used for Omnibank production. This automated process allows for the rapid identification of targeted events. It is important that unlike SA β geo, PGKpuroSD does not require expression of the targeted gene in order to provide positive selection. In addition, VICTR 20 provides 2 potential positive selectable markers (puro and neo). The use of two selectable markers, when a gene is expressed, provides a means to increase the

targeting efficiency by requiring both selectable markers to function which is much more remote a possibility than having one selectable marker function unless there is a targeted event. The addition of a negative selection cassette to 5 these vectors would only increase their targeting efficiency.

An additional feature that may be incorporated into the presently described vectors includes the use of recombinase recognition sequences. Bacteriophage P1 Cre recombinase and flp recombinase from yeast plasmids are two examples of 10 site-specific DNA recombinase enzymes which cleave DNA at specific target sites (loxP sites for cre recombinase and frt sites for flp recombinase) and catalyze a ligation of this DNA to a second cleaved site. When a piece of DNA is flanked by 2 loxP or frt sites (e.g., recombinase control elements) 15 in the same orientation, the corresponding recombinase will cause the removal of the intervening DNA sequence. When a piece of DNA is flanked by loxP or frt sites in an indirect orientation, the corresponding recombinase will essentially activate the control elements to cause the intervening DNA to 20 be flipped into the opposite orientation. These recombinases provide powerful approaches for manipulating DNA *in situ*.

Recombinases have important applications for gene trapping and the production of a library of trapped genes. When constructs containing PGKpuroSD are used to trap genes, 25 the fusion transcript between puromycin and sequences of the trapped gene could result in some level of protein expression from the trapped gene if translational reinitiation occurs. Another important issue is that several reports suggest that the PGK promoter can affect the expression of nearby genes. 30 These effects may make it difficult to determine gene function after a gene trap event since one could not discern whether a given phenotype is associated with the inactivation of a gene, or the transcription of nearby genes. Both potential problems are solved by exploiting recombinase 35 activity. When PGKpuroSD is flanked by loxP, frt, or any other recombinase sites in the same orientation, the addition of the corresponding recombinase will result in the removal

of PGKpuroSD. In this way, effects caused by PGKpuroSD fusion transcripts, or the PGK promoter, are avoided.

Accordingly, a vector that may be particularly useful for the practice of the present invention is VICTR 20. This vector replaces the terminal exon of VICTR 5 with a splice acceptor located upstream from the β geo gene which can be used for both LacZ staining and antibiotic selection. The fusion gene possesses its own initiator methionine and an internal ribosomal entry site (IRES) for efficient translation initiation. In addition, the PGK promoter and puromycin-splice donor sequences have been flanked by lox P recombination sites. This allows for the ability to both remove and introduce sequences at the integration site and is of potential value with regard to the manipulation of regions proximal to trapped target genes (Barinaga, Science 265:26-8, 1994). While this particular vector includes lox P recombination sites, the present invention is in no way limited to the use of this specific recombination site (Akagi et al., Nucleic Acids Res 25:1766-73, 1997).

Another very important use of recombinases is to produce mutations that can be made tissue-specific and/or inducible. In the presently described vectors, the SA β geo or SAIRES β geo component provides the mutagenic function by "trapping" the normal splicing from preceding exons. If the SA β geo is flanked by inverted loxP, frt, or any other recombinase sites, the addition of the corresponding recombinase results in the flipping of the SA β geo sequence so that it no longer prevents the normal splicing of the cellular gene into which it is integrated. To make a gene trap tissue-specific or inducible one could produce the trap with SA β geo in the reverse orientation and then provide recombinase activity only at the time and place where one wishes to remove the gene function. The use of tissue-specific or inducible recombinase constructs allows one to choose when and where one removes, or activates, the function of the targeted gene.

One method for practicing the inducible forms of recombinase mediated gene expression involves the use of

vectors that use inducible or tissue specific promoter/operator elements to express the desired recombinase activity. The inducible expression elements are preferably operatively positioned to allow the inducible control or
5 activation of expression of the desired recombinase activity. Examples of such inducible promoters or control elements include, but are not limited to, tetracycline, metallothionine, ecdysone, and other steroid-responsive promoters, rapamycin responsive promoters, and the like (No
10 et al., Proc Natl Acad Sci USA 93:3345-51, 1996; Furth et al., Proc Natl Acad Sci USA 91:9302-6, 1994). Additional control elements that can be used include promoters requiring specific transcription factors such as viral, particularly HIV, promoters. Vectors incorporating such promoters would
15 only express recombinase activity in cells that express the necessary transcription factors.

The incorporation of recombinase sites into the gene trapping vectors highlights the value of using the described gene trap vectors to deliver specific DNA sequence elements
20 throughout the genome. Although a variety of vectors are available for placing sequences into the genome, the presently described vectors facilitate both the insertion of the specific elements, and the subsequent identification of where sequence has inserted into the cellular chromosome.
25 Additionally, the presently described vectors may be used to place recombinase recognition sites throughout the genome. The recombinase recognition sites could then be used to either remove or insert specific DNA sequences at predetermined locations.

30 Moreover, the described gene trap vectors can also be used to insert regulatory elements throughout the genome. Recent work has identified a number of inducible or repressible systems that function in the mouse. These include the rapamycin, tetracycline, ecdysone,
35 glucocorticoid, and heavy metal inducible systems. These systems typically rely on placing DNA elements in or near a promoter. An inducible or repressible transcription factor

that can identify and bind to the DNA element may also be engineered into the cells. The transcription factor will specifically bind to the DNA element in either the presence or absence of a ligand that binds to the transcription factor
5 and, depending on the structure of the transcription factor, it will either induce or repress the expression of the cellular gene into which the DNA elements have been inserted. The ability to place these inducible or repressible elements throughout the genome would increase the value of the library
10 by adding the potential to regulate the expression of the trapped gene.

The vectors described also have important applications for the overexpression of genes or portions of genes to select for phenotypic effects. Currently, overexpression of
15 cDNA libraries to look for genes or parts of genes with specific functions is a common practice. One example would be to overexpress genes or portions of genes to look for expression that causes loss of contact inhibition for cell growth as determined by growth in soft agar. This would
20 allow the identification of genes or portions of genes that can act as oncogenes. Simple modifications of VICTR 20 would allow it to be used for these applications. For example, the addition of an internal ribosome entry site (IRES) 3' to the puromycin selectable marker and before the SD sequence, would
25 result in the overexpression of sequences from the trapped downstream exons. In addition, the IRES could be modified by, for example, the addition of one or two nucleotides such that there could be 3 basic vectors that would allow expression of trapped exons in all three reading frames. In
30 this way, genes could be trapped throughout the genome resulting in overexpression of genes, or portions thereof, to examine the cellular function of the trapped genes. This identification of function could be done by selecting for the function of interest (i.e., growth in soft agar could result
35 from the overexpression of potentially oncogenic genes). This technique would allow for the screening or selection of large numbers of genes, or portions thereof, by

overexpressing the genes and identifying cells displaying the phenotypes of interest. Additional assays could, for example, identify candidate tumor suppressor genes based on their ability, when overexpressed, to prevent growth in soft agar.

Given the fact that expression pattern information can provide insight into the possible functions of genes mutated by the current methods, another LTR vector, VICTR 6, has been constructed in a manner similar to VICTR 5 except that the terminal exon has been replaced with either a gene coding for β -galactosidase (β gal) or a fusion between β -gal and neomycin phosphotransferase (β geo), each proceeded by a splice acceptor and followed by a polyadenylation signal. Endogenous gene expression and splicing of these markers into cellular transcripts and translation into fusion proteins will allow for increased mutagenicity as well as the delineation of expression through Lac Z staining.

An additional vector, VICTR 12, incorporates two separate selectable markers for the analysis of both integration sites and trapped genes. One selectable marker (e.g. puro) is similar to that for VICTRs 3 through 5 in that it contains a promoter element at its 5' end and a splice donor sequence 3'. This gene cassette is located in the LTRs of the retroviral vector. The other marker (neo) also contains a promoter element but has a polyadenylation signal present at the 3' end of the coding sequence and is positioned between the viral LTRs. Both selectable markers contain an initiator ATG for proper translation. The design of VICTR 12 allows for the assessment of absolute titer as assayed by the number of colonies resistant to antibiotic selection for the constitutively expressed marker possessing a polyadenylation signal. This titer can then be compared to that observed for gene-trapping and stable expression of the resistance marker flanked at its 3' end by a splice donor. These numbers are important for the calculation of gene trapping frequency in the context of both nonspecific binding by retroviral integrase and directed binding by chimeric

integrase fusions. In addition, it provides an option to focus on the actual integration sites through infection and selection for the marker containing the polyadenylation signal. This eliminates the need for the fusion protein binding to occur upstream and in the proximity of the target gene. Theoretically, any transcription factor binding sites present within the genome are targets for proximal integration and subsequent antibiotic resistance. Analysis of sequences flanking the LTRs of the retroviral vector should reveal canonical factor binding sites. In addition, by including the promoter/splice donor design of VICTR 3, gene-trapping abilities are retained in VICTR 12.

VICTR A is a vector which does not contain gene trapping constructs but rather a selectable marker possessing all of the required entities for constitutive expression including, but not limited to, a promoter element capable of driving expression in eukaryotic cells and a polyadenylation and transcriptional terminal signal. Similar to VICTR 12, downstream gene trapping is not necessary for successful selection using VICTR A. This vector is intended solely to select for successful integrations and serves as a control for the identification of transcription factor binding sites flanking the integrant as mentioned above.

Finally, VICTR B is similar to VICTR A in that it comprises a constitutively expressed selectable marker, but it also contains the bacterial β -lactamase ampicillin resistance selectable marker and a ColE1 origin of replication. These entities allow for the rapid cloning of sequences flanking the long terminal repeats through restriction digestion of genomic DNA from infected cells and ligation to form plasmid molecules which can be rescued by bacterial transformation, and subsequently sequenced. This vector allows for the rapid analysis of cellular sequences that contain putative binding sites for the transcription factor of interest.

Other vector designs contemplated by the present invention are engineered to include an inducible regulatory

elements such as tetracycline, ecdysone, and other steroid-responsive promoters (No et al., Proc Natl Acad Sci USA 93:3345-51, 1996; Furth et al., Proc Natl Acad Sci USA 91:9302-6, 1994). These elements are operatively positioned
5 to allow the inducible control of expression of either the selectable marker or endogenous genes proximal to site of integration. Such inducibility provides a unique tool for the regulation of target gene expression.

All of the gene trap vectors of the VICTR series, with
10 the exception of VICTRs A and B, are designed to form a fusion transcript between vector encoded sequence and the trapped target gene. All of the flanking exons may be sequenced according to the methods described in the following section. To facilitate sequencing, specific sequences are
15 engineered onto the ends of the selectable marker (e.g., puromycin coding region). Examples of such sequences include, but are not limited to unique sequences for priming PCR, and sequences complementary to standard M13 sequencing primers. Additionally, stop codons are added in all three
20 reading frames to ensure that no anomalous fusion proteins are produced. All of the unique 3' primer sequences are immediately followed by a synthetic 9 base pair splice donor sequence. This keeps the size of the exon comprising the selectable marker at a minimum to ensure proper splicing, and
25 positions the amplification and sequencing primers immediately adjacent to the flanking trapped exons to be sequenced as part of the generation of the collection of cells representing mutated transcription factor targets.

Since a cryptic splice donor sequence is found in the
30 inverted LTRs, this cryptic splice donor sequence has been removed from the VICTR vectors by site specific mutagenesis. It was deemed necessary to remove this splice donor so that it would not affect trapping associated splicing events.

When any members of the VICTR series are packaged into
35 infectious virus, the direction of transcription of the selectable marker is opposite to that of the direction of the normal transcription of the retrovirus. The reason for this

organization is that the regulatory elements such as the polyadenylation signal, the splice sites and the promoter elements found in the various members of the VICTR series can interfere with the transcription of the retroviral genome in the packaging cell line. This potential interference may significantly reduce retroviral titers.

Although specific gene trapping vectors have been discussed at length above, the invention is by no means to be limited to such vectors. Several other types of vectors that may also be used to incorporate relatively small engineered exons into a target cell transcripts include, but are not limited to, adenoviral vectors, adenoassociated virus vectors, SV40 based vectors, and papilloma virus vectors. Additionally, DNA vectors may be directly transferred into the target cells using any of a variety of biochemical or physical means such as lipofection, chemical transfection, retrotransposition, electroporation, and the like.

Although, the use of specific selectable markers has been disclosed and discussed herein, the present invention is in no way limited to the specifically disclosed markers. Additional markers (and associated antibiotics) that are suitable for either positive or negative selection of eukaryotic cells are disclosed, inter alia, in Sambrook et al. (1989) Molecular Cloning Vols. I-III, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, and Current Protocols in Molecular Biology (1989) John Wiley & Sons, all Vols. and periodic updates thereof, as well as Table I of U.S. Patent No. 5,464,764 issued November 7, 1995, the entirety of which is herein incorporated by reference. Any of the disclosed markers, as well as others known in the art, may be used to practice the present invention.

5.2. The Analysis of Mutated Genes and Transcripts

The presently described invention allows for large-scale genetic analysis of the genomes of any organism for which there exists cultured cell lines. The Library may be constructed from any type of cell that can be transfected by

standard techniques or infected with recombinant retroviral vectors.

Where mouse ES cells are used, then the Library becomes a genetic tool able to completely represent mutations in
5 essentially every gene of the mouse genome. Since ES cells can be injected back into a blastocyst and become incorporated into normal development and ultimately the germ line, the cells of the Library effectively represent a complete panel of mutant transgenic mouse strains (see
10 generally, U.S. Patent No. 5,464,764 issued November 7, 1995, herein incorporated by reference).

A similar methodology may be used to construct virtually any non-human transgenic animal (or animal capable of being rendered transgenic). Such nonhuman transgenic animals may
15 include, for example, transgenic pigs, transgenic rats, transgenic rabbits, transgenic cattle, transgenic goats, and other transgenic animal species, particularly mammalian species, known in the art. Additionally, bovine, ovine, and porcine species, other members of the rodent family, e.g.
20 rat, as well as rabbit and guinea pig and non-human primates, such as chimpanzee, may be used to practice the present invention.

Transgenic animals produced using the presently described library and/or vectors are useful for the study of
25 basic biological processes and diseases including, but not limited to, aging, cancer, autoimmune disease, immune disorders, alopecia, glandular disorders, inflammatory disorders, diabetes, arthritis, high blood pressure, atherosclerosis, cardiovascular disease, pulmonary disease,
30 degenerative diseases of the neural or skeletal systems, Alzheimer's disease, Parkinson's disease, asthma, developmental disorders or abnormalities, infertility, epithelial ulcerations, and microbial pathogenesis (a relatively comprehensive review of such pathogens is
35 provided, *inter alia*, in Mandell et al., 1990, "Principles and Practice of Infectious Disease" 3rd. ed., Churchill Livingstone Inc., New York, N.Y. 10036, herein incorporated

by reference). As such, the described animals and cells are particularly useful for the practice of functional genomics.

5.2.1. Constructing a Library of Individually Mutated Cell Clones

5 The vectors described in the previous section were used to infect (or transfect) cells in culture, for example, mouse embryonic stem (ES) cells. Gene trap insertions were initially identified by antibiotic resistance (e.g., puromycin). Individual clones (colonies) were moved
10 from a culture dish to individual wells of a multi-welled tissue culture plate (e.g. one with 96 wells). From this platform, the clones were be duplicated for storage and subsequent analysis. Each multi-well plate of clones was
15 then processed by molecular biological techniques described in the following section in order to derive sequence of the gene that has been mutated. This entire process is presented schematically in Figure 4 (described below).

20 5.2.2. Identifying and Sequencing the Tagged Genes in the Library.

The relevant nucleic acid (and derived amino acid sequence information) will largely be obtained using PCR-based techniques that rely on knowing part of the sequence of the fusion transcripts (see generally, Frohman et
25 al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85(23):8998-9000, and U.S. Patents Nos. 4,683,195 to Saiki et al., and 4,683,202 to Mullis, which are herein incorporated by reference). Typically, such sequences are encoded by the foreign exon containing the selectable marker. The procedure
30 is represented schematically in Figure 2 (3' RACE). Although each step of the procedure may be done manually, the procedure is also designed to be carried out using robots that can deliver reagents to multi well culture plates (e.g., but not limited to, 96-well plates).

35 The first step generates single stranded complementary DNA which is used in the PCR amplification reaction (Figure

2). The RNA substrate for cDNA synthesis may either be total cellular RNA or an mRNA fraction; preferably the latter. mRNA was isolated from cells directly in the wells of the tissue culture dish. The cells were lysed and mRNA was bound 5 by the complementary binding of the poly-adenylate tail to a poly-thymidine-associated solid matrix. The bound mRNA was washed several times and the reagents for the reverse transcription (RT) reaction were added. cDNA synthesis in the RT reaction was initiated at random positions along the 10 message by the binding of a random sequence primer (RS). This RS primer has approximately 6-9 random nucleotides at the 3' end to bind sites in the mRNA to prime cDNA synthesis, and a 5' tail sequence of known composition to act as an anchor for PCR amplification in the next step. There is 15 therefore no specificity for the trapped message in the RT step. Alternatively, a poly-dT primer appended with the specific sequences for the PCR may be used. Synthesis of the first strand of the cDNA initiates at the end of each trapped gene. At this point in the procedure, the bound mRNA may be 20 stored (at between about -70° C and about 4° C) and reused multiple times. Such storage is a valuable feature where one subsequently desires to analyze individual clones in more detail. The bound mRNA may also be used to clone the entire transcript using PCR-based protocols.

25 Specificity for the trapped, fusion transcript is introduced in the next step, PCR amplification. The primers for this reaction are complementary to the anchor sequence of the RS primer and to the selectable marker. Double stranded fragments between a fixed point in the selectable marker gene 30 and various points downstream in the appended transcript sequence are amplified. It is these fragments which will become the substrates for the sequencing reaction. The various end-points along the transcript sequence were determined by the binding of the random primer during the RT 35 reaction. These PCR products were diluted into the sequencing reaction mix, denatured and sequenced using a primer specific for the splice donor sequences of the gene

trap exon. Although, standard radioactively labeled nucleotides may be used in the sequencing reactions, sequences will typically be determined using standard dye terminator sequencing in conjunction with automated
5 sequencers (e.g., ABI sequencers and the like).

Several fragments of various sizes may serve as substrates for the sequencing reactions. This is not a problem since the sequencing reaction proceeds from a fixed point as defined by a specific primer sequence. Typically,
10 approximately 200 nucleotides of sequence were obtained for each trapped transcript. For the PCR fragments that are shorter than this, the sequencing reaction simply 'falls off' the end. Sequences further 3' were then covered by the longer fragments amplified during PCR. One problem is
15 presented by the anchor sequences 'S' derived from the RS primer. When these are encountered during the sequencing of smaller fragments, they register as anomalous dye signals on the sequencing gels. To circumvent this potential problem, a restriction enzyme recognition site is included in the S
20 sequence. Digestion of the double stranded PCR products with this enzyme prior to sequencing eliminates the heterologous S sequences.

5.2.3. Identifying the Tagged Genes by Chromosomal Location

25 Any individually tagged gene may also be identified by PCR using chromosomal DNA as the template. To find an individual clone of interest in the Library arrayed as described above, genomic DNA is isolated from the pooled
30 clones of ES cells as presented in Figure 3. One primer for the PCR is anchored in the gene trap vector, e.g., a puro exon-specific oligonucleotide. The other primer is located in the genomic DNA of interest. This genomic DNA primer may consist of either (1) DNA sequence that corresponds to the
35 coding region of the gene of interest, or (2) DNA sequence from the locus of the gene of interest. In the first case, the only way that the two primers used may be juxtaposed to

give a positive PCR results (e.g., the correct size double-stranded DNA product) is if the gene trap vector has inserted into the gene of interest. Additionally, degenerate primers may be used, to identify and isolate related genes of interest. In the second case, the only way that the two primers used may be juxtaposed to provide the desired PCR result is if the gene trap vector has inserted into the region of interest that contains the primer for the known marker.

For example, if one wishes to obtain ES cell clones from the library that contain mutated genes located in a certain chromosomal position, PCR primers are designed that correspond to the puro gene (the puro-anchored primer) and a primer that corresponds to a marker known to be located in the region of interest. Several different combinations of marker primers and primers that are located in the region of interest may also be used to obtain optimum results. In this manner, the mutated genes are identified by virtue of their location relative to sets of known markers. Genes in a particular chromosomal region of interest could therefore be identified. The marker primers could also be designed correspond to sequences of known genes in order to screen for mutations in particular genes by PCR on genomic DNA templates. While this method is likely to be less informative than the RT-PCR strategy described below, this technique would be useful as a alternative strategy to identify mutations in known genes. In addition, primers that correspond to sequence of known genes could be used in PCR reactions with marker-specific primers in order to identify ES cell clones that contain mutations in genes proximal to the known genes. The sensitivity of detection is adequate to find such events when positive clones are subsequently identified as described below in the RT-PCR strategy.

5.3. A Sequence Database Identifies Genes Mutated in the Library.

Using the procedures described above, approximately 200

to about 600 bases of sequence from the cellular exons appended to the selectable marker exon (e.g., puro exon in VICTR vectors) may be identified. These sequences provide a means to identify and catalogue the genes mutated in each
5 clone of the Library. Such a database provides both an index for the presently disclosed libraries, and a resource for discovering novel genes. Alternatively, various comparisons can be made between the Library database sequences and any other sequence database as would be familiar to those
10 practiced in the art.

The novel utility of the Library lies in the ability for a person to search the Library database for a gene of interest based upon some knowledge of the nucleic acid or amino acid sequence. Once a sequence is identified, the
15 specific clone in the Library can be accessed and used to study gene function. This is accomplished by studying the effects of the mutation both *in vitro* and *in vivo*. For example, cell culture systems and animal models (i.e., transgenic animals) may be directly generated from the cells
20 found in the Library as will be familiar to those practiced in the art.

Additionally, the sequence information may be used to generate a highly specific probe for isolating both genomic clones from existing data bases, as well as a full length
25 cDNA. Additionally, the probe may be used to isolate the homologous gene from sufficiently related species, including humans. Once isolated, the gene may be over expressed, or used to generate a targeted knock-out vector that may be used to generate cells and animals that are homozygous for the
30 mutation of interest. Such animals and cells are deemed to be particularly useful as disease models (i.e., cancer, genetic abnormalities, AIDS, etc.), for developmental study, to assay for toxin susceptibility or the efficacy of therapeutic agents, and as hosts for gene delivery and
35 therapy experiments (e.g., experiments designed to correct a specific genetic defect *in vivo*).

5.4. Accessing Clones in the Library by a Pooling and Screening Procedure.

An alternative method of accessing individual clones is by searching the Library database for sequences in order to isolate a clone of interest from pools of library clones. The Library may be arrayed either as single clones, each with different insertions, or as sets of pooled clones. That is, as many clones as will represent insertions into essentially every gene in the genome are grown in sets of a defined number. For example, 100,000 clones can be arrayed in 2,000 sets of 50 clones. This can be accomplished by titrating the number of VICTR retroviral particles added to each well of 96-well tissue culture plates. Two thousand clones will fit on approximately 20 such plates. The number of clones may be dictated by the estimated number of genes in the genome of the cells being used. For example, there are approximately 100,000 genes in the genome of mouse ES cells. Therefore, a Library of mutations in essentially every gene in the mouse genome may be arrayed onto 20 96-well plates.

To find an individual clone of interest in the Library arrayed in this manner, reverse transcription-polymerase chain reactions (RT-PCR) are performed on mRNA isolated from pooled clones as presented in Figure 4. One primer for RT-PCR is anchored in the gene trap vector, i.e. a puro exon-specific oligonucleotide. The other primer is located in the cDNA sequence of a gene of interest. The only way that these two sequences can be juxtaposed to give a positive RT-PCR result (i.e. double stranded DNA fragment visible by agarose gel electrophoresis, as will be familiar to anyone practiced in the art) is by being present in a transcript from a gene trap event occurring in the gene of interest.

For example, if one wishes to obtain an ES cell clone with a mutation in the p53 gene, PCR primers are designed that correspond to the puro and p53 genes. If a VICTR trapping vector integrates into the p53 locus and results in the formation of a fusion mRNA, this mRNA may be detected by RT-PCR using these specifically designed primer pairs. The

sensitivity of detection is adequate to find such an event when positive cells are mixed with a large background of negative cells. The individual positive clones are subsequently identified by first locating the pool of 50 clones in which it resides. This process is described in Figure 5. The positive pool, once identified, is subsequently plated at limiting dilution (approximately 0.3 cells/well) such that individual clones may be isolated. To find the one positive event in 50 clones represented by this pool, individual clones are isolated and arrayed on a 96-well plate. By pooling in columns and rows, the positive well containing the positive clone can be identified with relatively few RT-PCR reactions.

In addition to RT-PCR, the pools may be screened by hybridization techniques (see generally Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual 2nd edition, Cold Spring Harbor Press, Cold Spring Harbor, and Current Protocols in Molecular Biology, 1995, Ausubel et al. eds., John Wiley and Sons). Specific PCR fragments are generated from the mutated genes essentially as described above for the sequencing protocols of the individual clones (first-strand synthesis using RT primed by a random or oligo dT primer that is appended to a specific primer binding site). The gene trap DNA is amplified from the primer sets in the puro gene and the specific sequences appended to the RT primer. If this were done with pools, the resulting pooled set of amplified DNA fragments could be arrayed on membranes and probed by radioactive, or chemically or enzymatically labeled, hybridization probes specific for a gene of interest. A positive radioactive result indicates that the gene of interest has been mutated in one of the clones of the positively-labeled pool. The individual positive clone is subsequently identified by PCR or hybridization essentially as outlined above.

Alternatively, a similar strategy may be used to identify the clone of interest from multiple plates, or any scheme where a two or three dimensional array (e.g., columns

and rows) of individual clones are pooled by row or by column. For example, 96 well plates of individual clones may be arranged adjacent to each other to provide a larger (or virtual/figurative) two dimensional grid (e.g., four plates may be arranged to provide a net 16x24 grid), and the various rows and columns of the larger grid may be pooled to achieve substantially the same result.

Similarly, plates may simply be stacked, literally or figuratively, or arranged into a larger grid and stacked to provide three dimensional arrays of individual clones. Representative pools from all three planes of the three dimensional grid may then be analyzed, and the three positive pools/planes may be aligned to identify the desired clone. For example, ten 96 well plates may be screened by pooling the respective rows and columns from each plate (a total of 20 pools) as well as pooling all of the clones on each specific plate (10 additional pools). Using this method, one may effectively screen 960 clones by performing PCR on only 30 pooled samples.

The example provided below is merely illustrative of the subject invention. Given the level of skill in the art, one may be expected to modify any of the above or following disclosure to produce insubstantial differences from the specifically described features of the present invention. As such, the following example is provided solely by way of illustration and is not included for the purpose of limiting the invention in any way whatsoever.

6.0. EXAMPLES

6.1. Use of VICTR Series Vectors to Construct a Mouse ES cell Gene Trap Library

VICTR 3 was used to gather a set of gene trap clones. A plasmid containing the VICTR 3 cassette was constructed by conventional cloning techniques and designed to employ the features described above. Namely, the cassette contained a PGK promoter directing transcription of an exon that encodes the puro marker and ends in a canonical splice donor

sequence. At the end of the puromycin exon, sequences were added as described that allow for the annealing of two nested PCR and sequencing primers. The vector backbone was based on pBluescript KS+ from Stratagene Corporation.

5 The plasmid construct linearized by digestion with Sca I which cuts at a unique site in the plasmid backbone. The plasmid was then transfected into the mouse ES cell line AB2.2 by electroporation using a BioRad Genepulser apparatus. After the cells were allowed to recover, gene trap clones
10 were selected by adding puromycin to the medium at a final concentration of 3 μ g/mL. Positive clones were allowed to grow under selection for approximately 10 days before being removed and cultured separately for storage and to determine the sequence of the disrupted gene.

15 Total RNA was isolated from an aliquot of cells from each of 18 gene trap clones chosen for study. Five micrograms of this RNA was used in a first strand cDNA synthesis reaction using the "RS" primer. This primer has unique sequences (for subsequent PCR) on its 5' end and nine
20 random nucleotides or nine T (thymidine) residues on its 3' end. Reaction products from the first strand synthesis were added directly to a PCR with outer primers specific for the engineered sequences of puromycin and the "RS" primer. After amplification, an aliquot of reaction products were subject
25 to a second round of amplification using primers internal, or nested, relative to the first set of PCR primers. This second amplification provided more reaction product for sequencing and also provided increased specificity for the specifically gene trapped DNA.

30 The products of the nested PCR were visualized by agarose gel electrophoresis, and seventeen of the eighteen clones provided at least one band that was visible on the gel with ethidium bromide staining. Most gave only a single band which is an advantage in that a single band is generally
35 easier to sequence. The PCR products were sequenced directly after excess PCR primers and nucleotides were removed by filtration in a spin column (Centricon-100, Amicon). DNA was

added directly to dye terminator sequencing reactions (purchased from ABI) using the standard M13 forward primer a region for which was built into the end of the puro exon in all of the PCR fragments. Thirteen of the seventeen clones 5 that gave a band after the PCR provided readable sequence. The minimum number of readable nucleotides was 207 and some of the clones provided over 500 nucleotides of useful sequence.

Sample data from this set of clones is presented in 10 Figure 6. Only a portion of sequence (nucleotide or putative amino acid) for 9 Library clones obtained by the methods described in this invention are presented. Under each sequence fragment in the figure is aligned a homologous sequence that was identified using the BLAST (basic local 15 alignment search tool) search algorithm (Altschul et al., 1990, J. Mol. Biol. 215:403-410).

In addition to known sequences, many new genes were also identified. Each of these sequences is labeled "OST" for "Omnibank Sequence Tags." OMNIBANK™ shall be the trademark 20 name for the Libraries generated using the disclosed technology.

These data demonstrate that the VICTR series vectors may efficiently trap genes, and that the procedures used to obtain sequence are reliable. With simple optimization of 25 each step, it is presently possible to mutate every gene in a given population of cells, and obtain sequence from each of these mutated genes. The sample data provided in this example represents a small fraction of an entire Library. By simply performing the same procedures on a larger scale (with 30 automation) a Library may be constructed that collectively comprises and indexes mutations in essentially every gene in the genome of the target cell.

Additional studies have used both VICTR 3 and VICTR 20. Like VICTR 3, VICTR 20 is exemplary of a family of vectors 35 that incorporate two main functional units: a sequence acquisition component having a strong promoter element (phosphoglycerate kinase 1) active in ES cells that is fused

to the puromycin resistance gene coding sequence which lacks a polyadenylation sequence but is followed by a synthetic consensus splice donor sequence (PGKpuroSD); and 2) a mutagenic component that incorporates a splice acceptor sequence fused to a selectable, colorimetric marker gene and followed by a polyadenylation sequence (for example, SA β geopA or SAIRES β geopA). Also like VICTR 3, stop codons have been engineered into all three reading frames in the region between the 3' end of the selectable marker and the splice donor site. A diagrammatic description of structure and functions of VICTRs 3 and 20 is provided in Figure 7.

When VICTRs 3 and 20 were used in the commercial scale application of the presently disclosed invention, over 3,000 mutagenized ES cell clones were rapidly engineered and obtained. Sequence analysis obtained from these clones has identified a wide variety of both previously identified and novel sequences. A representative sampling of previously known genes that were identified using the presently described methods is provided in Figure 8. The power of the presently described invention as a genomics resource becomes apparent when one considers that the genes listed in Figure 8 were obtained and identified in less than a year whereas the references associated with the identification of the known genes span a period of roughly two decades. More importantly, the majority of the sequences thus far identified are novel, and, because of the functional aspects of the presently described ES cell system, the cellular and developmental functions of these novel sequences can be rapidly established.

30

7.0. Reference to Microorganism Deposits

The following plasmids have been deposited at the American Type Culture Collection (ATCC), Rockville, MD, USA, under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and Regulations thereunder (Budapest Treaty) and are thus maintained and made available according

to the terms of the Budapest Treaty. Availability of such plasmids is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent
5 laws.

The deposited cultures have been assigned the indicated ATCC deposit numbers:

	<u>Plasmid</u>	<u>ATCC No.</u>
	plex	97748
10	pExonII	97749
	ppuro7	97750
	ppuro5	97751
	ppuro11	97752
	ppuro10	97753

All publications and patents mentioned in the above specification are herein incorporated by reference. Various
15 modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be
20 understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the above-described modes for carrying out the invention which are obvious to those skilled in the field of molecular biology or related fields are intended to be
25 within the scope of the following claims.

30

35

MICROORGANISMS	
Optional Sheet in connection with the microorganism referred to on page <u>40</u> , lines <u>5-25</u> of the description *	
A. IDENTIFICATION OF DEPOSIT * Further deposits are identified on an additional sheet *	
Name of depositary institution * American Type Culture Collection	
Address of depositary institution (including postal code and country) * 12301 Parklawn Drive Rockville, MD 20852 US	
Date of deposit * <u>October 9, 1996</u> Accession Number * <u>97748</u>	
B. ADDITIONAL INDICATIONS * (leave blank if not applicable). This information is continued on a separate attached sheet	
C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE * (if the indications are not all designated States)	
D. SEPARATE FURNISHING OF INDICATIONS * (leave blank if not applicable) The indications listed below will be submitted to the International Bureau later * (Specify the general nature of the indications e.g., "Accession Number of Deposit")	
E. <input type="checkbox"/> This sheet was received with the International application when filed (to be checked by the receiving Office)	
<div style="text-align: right;">_____ (Authorized Officer)</div>	
<input type="checkbox"/> The date of receipt (from the applicant) by the International Bureau *	
was _____ (Authorized Officer)	

Form PCT/RO/134 (January 1981)

International Application No: PCT/ /

Form PCT/RO/134 (cont.)

American Type Culture Collection

12301 Parklawn Drive
Rockville, MD 20852
US

<u>Accession No.</u>	<u>Date of Deposit</u>
97749	October 9, 1996
97750	October 9, 1996
97751	October 9, 1996
97752	October 9, 1996
97753	October 9, 1996

CLAIMSWhat is claimed is:

1. A library of cultured eucaryotic cells made by a process comprising the steps of:
 - 5 a) treating a first group of cells to stably integrate a first vector that mediates the splicing of a foreign exon internal to a cellular transcript;
 - b) treating a second group of cells to stably integrate a second vector that mediates the splicing of a foreign exon
10 5' to an exon of a cellular transcript; and
 - c) selecting for transduced cells that express the products encoded by the foreign exons.
2. A library according to claim 1 wherein said treating
15 is transfection.
3. A library according to claim 1 wherein said treating is by infection.
- 20 4. A library according to claim 1 wherein said treating is by retrotransposition.
5. A library according to any one of claims 1 through 4 wherein said cells are animal cells.
25
6. A library according to claim 5 wherein said animal is mammalian.
7. A library according to claim 6 wherein said cells
30 are rodent cells.
8. The use of a mutated cell from a library according to claim 6 to generate a non-human transgenic animal.
- 35 9. A vector for replacing the 3' end of an animal cell transcript with a foreign exon, comprising:
 - a) a selectable marker;

- b) a splice acceptor site operatively positioned 5' to the initiation codon of said selectable marker;
- c) a polyadenylation site operatively positioned 3' to said selectable marker;
- 5 d) said vector not comprising a promoter element operatively positioned 5' of the coding region of said selectable marker; and
- e) said vector not comprising a splice donor sequence operatively positioned between the 3' end of the
10 coding region of said selectable marker and said polyadenylation site.

10. A vector for inserting foreign mutagenic polynucleotide sequence internal to animal cell transcripts,
15 comprising:

- a) a foreign exon;
- b) a splice acceptor sequence operatively positioned 5' to the foreign exon;
- c) a splice donor site operatively positioned 3' to
20 said foreign exon;
- d) a sequence comprising a nested set of stop codons in each of the three reading frames located between the 3' end of said foreign exon and said splice donor site;
- 25 e) said vector not comprising a polyadenylation site operatively positioned 3' to said foreign exon; and
- f) said vector not comprising a promoter element operatively positioned 5' to the coding region of said foreign exon.

30

11. A vector for attaching a foreign exon upstream from the 3' end of an animal cell transcript, comprising:

- a) a selectable marker;
- b) a promoter element operatively positioned 5' to
35 said selectable marker;
- c) a splice donor site operatively positioned 3' to said selectable marker; and

- d) said vector not comprising a transcription terminator or polyadenylation site operatively positioned relative to the coding region of said selectable marker; and
- 5 e) said vector not comprising a splice acceptor site operatively positioned between said promoter element and the initiation codon of said selectable marker.

10 12. A vector according to claim 11 wherein said vector additionally comprises a foreign mutagenic polynucleotide sequence located upstream from said promoter.

15 13. A vector according to claim 12 wherein said vector additionally comprises a splice acceptor operatively positioned upstream from said foreign mutagenic polynucleotide sequence.

20 14. A vector according to claim 13 wherein said foreign mutagenic polynucleotide sequence comprises a polyadenylation site.

25 15. A vector according to claim 14, wherein said foreign mutagenic polynucleotide sequence additionally comprises stop codons in all three reading frames.

16. A vector according to claim 12 in which a first recombinase recognition sequence is present upstream from said promoter and a second recombinase recognition sequence
30 is present downstream from said promoter.

17. A vector according to any one of claims 9, 10, or 11 wherein said vector is a viral vector.

35 18. A vector according to claim 17 wherein said viral vector is a retroviral vector.

19. The use of a vector according to claim 9 to produce a library of mutated animal cells.

20. The use of a vector according to claim 10 to produce mutated animal cells.

21. The use of a vector according to claim 11 to produce mutated animal cells.

22. The use of a vector according to claim 11 to effect homologous recombination in an animal cell.

23. A stably transduced animal cell that incorporates a vector according to claim 16.

15

24. A method of deleting a region of vector DNA from a cell according to claim 23, comprising:

- a) providing a recombinase activity to the cell; and
- b) selecting for cells that lack the desired region of vector DNA.

20

25. A method of adding a region of DNA to a cell according to claim 23, comprising:

- a) introducing the DNA to be added into the cell;
- a) providing a recombinase activity to the cell; and
- b) selecting for cells that incorporate the added DNA.

25

26. A method of effecting the inducible expression of a desired gene, comprising:

- a) providing a cell according to claim 23 with a recombinase gene that is expressed by an inducible promoter; and
- b) inducing said inducible promoter.

30

27. A method of gene discovery comprising:

35

- a) adding a foreign polynucleotide to a population of target cells such that the foreign

polynucleotide is inserted throughout the genomes of the target cells; and

b) activating control elements encoded by the foreign polynucleotides that activate or repress the expression of target cell genes that flank the integrated foreign polynucleotides, and identifying the regions of the target cell genome into which the foreign polynucleotides have integrated.

28. A library of cultured animal cells that stably integrate vectors according to claims 10 or 11.

15

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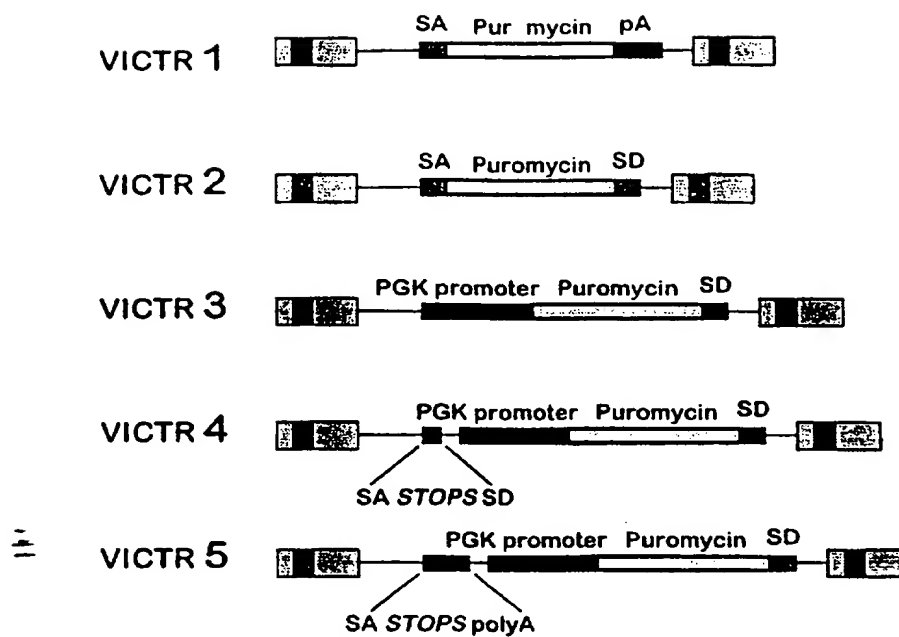


Figure 1

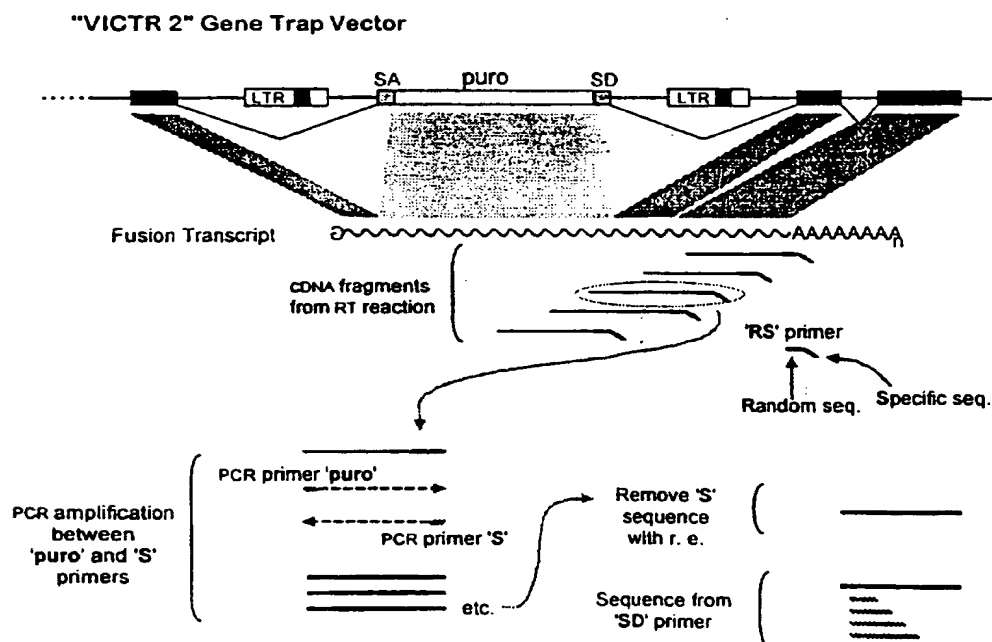


Figure 2

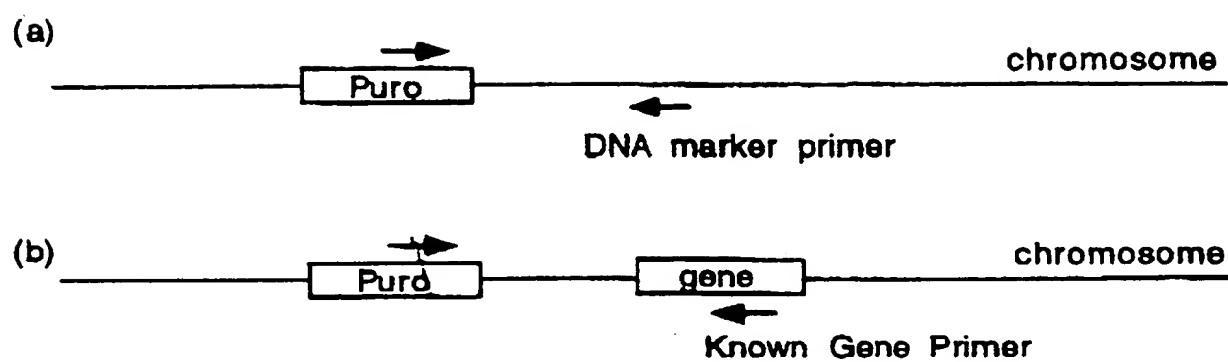


Figure 3

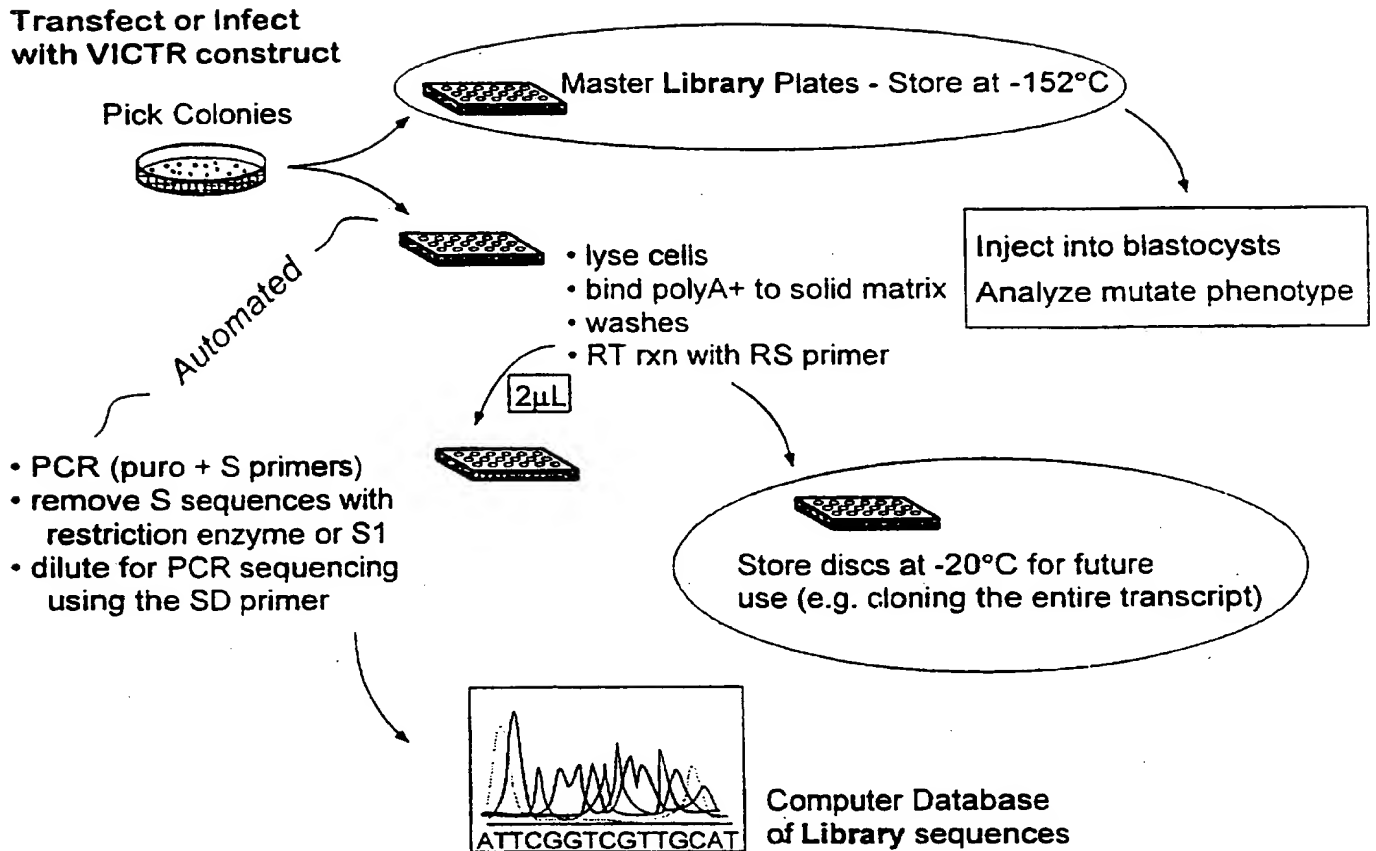
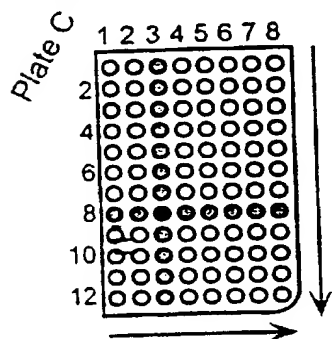


Figure 4

Identify Positive Pool

To screen all mouse genes (~100,000) with 5-fold redundancy would require about 50 plates of 96-wells (at 100 clones/well).



① Pool columns

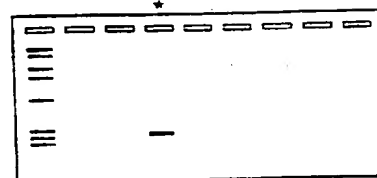
(100 clones/well)(12 wells) = 1200 clones per rxn.
(50 plates)(8 columns/plate) = 400 initial rxns.

Plate C, column 3 was positive.

② Pool positive rows only

8 rxns per positive plate

Row 8 was positive.

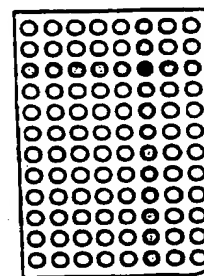


Identify Positive Clone

The pool on plate C, column 3, row 8 is thawed and plated as single clones:



① Pick 96 colonies into two 96-well plates



② Pool columns (16) and rows (24) for 40 rxns to identify positive clone.

Figure 5

OST1:	248	TTTATATAATATTAAATTGTTTACTGGGGTATATATGTGTGAAGAGGACTTCT	302
rat GABA rho3:	1547	TTTACATAATATTAAATTGTTTACTGGGGTATATATGTGTGAAGAGGACTTTT	1601
OST2:	56	ACCGTTGCGGAGGCTCAGTTTCTCAGATAGTACATCAGGTGTCATCGNTGTCAGAAAGT	115
mouse TCR-ATF1:	75	ACCGTTGCGGGGCTCAGTTTCTCAGATAGTACATCAGGTGTCATCGTTATCAGAAAGT	134
OST3:	58	GIGMHAGLHERDRKTVEELFXNCKVQVLIATSTLAWGVNFAHLVLIKSTEYDGTFR	237
		GIG+HHAGL ++DR +LF K+Q+LIATSTLAWGVN PAHLVLIKGT+++D K	
Yeast ORF G9365:	1430	GIGLHHAGLVQKDRSISHQLFQNKIQILIIATSTLAWGVNPAHLVLIKGTQFFDAKIEG	1489
OST4:	137	GCGCAGAAGTGGTCTGGAANTTTNTCCGCCNCCATCCAGTCTATTAAATTGTTGACNGGA	196
seq. from US			
patent 5470724:	166	GCGCAGAAGTGGTCTGCAACTTTATCCGCCTCCATCCAGTCTATTAAATTGTTGCCGGGA	225
OST5:	108	TCWIRLGT*RXVGASLEYEYIRAS	179
mouse wnt-5A		TCW++L R VG +L+ +Y A+	
protein precursor:	250	TCWLQLADFRKVGDALKEYDSAA	273
OST6:	78	CTTATATGGCTACGGCGGCTTCAACATCTCCATTACACCCAACACTACAGCGTGTCCAGGCT	137
human prolyl			
endopeptidase:	1407	CTTATATGGCTATGGCGGCTTCAACATATCCATCACACCCAACACTACAGTGTTCAGGCT	1466
OST7:	109	AAAGCATGTAGCAGTTGTAGGACACACTAGACGAGAGCACCAGATCTCATTGTGGGTGGT	168
mouse			
45S pre rRNA:	1604	AAAGCATGTAGCAGTTGTAGGACACACTAGACGAGAGCACCAGATCTCATTGTGGGTGGT	1663
OST8:	161	TGGATGCAGNCTACCACTGTGTGGCTGCCCTATTTTACCTCAGTGCCTCAGTTCGGAAG	220
rat MAL:	306	TGGATGCAGCCTACCACTGTGTGGCTGCCCTGTTTTACCTCAGTGCCTCAGTTCGGAAG	365
OST9:	103	ACCTGATTGTTATCCGTGGCCTGCAGAAGTCCAGAAAATACAGACCAAAGTCAACCAGTA	162
mouse malic enzyme:	1666	ACCTGATTGTTATCCGTGGCCTGCAGAAGTCCAGAAAATACAGACCAAAGTCAACCAGTA	1725

Figure 6

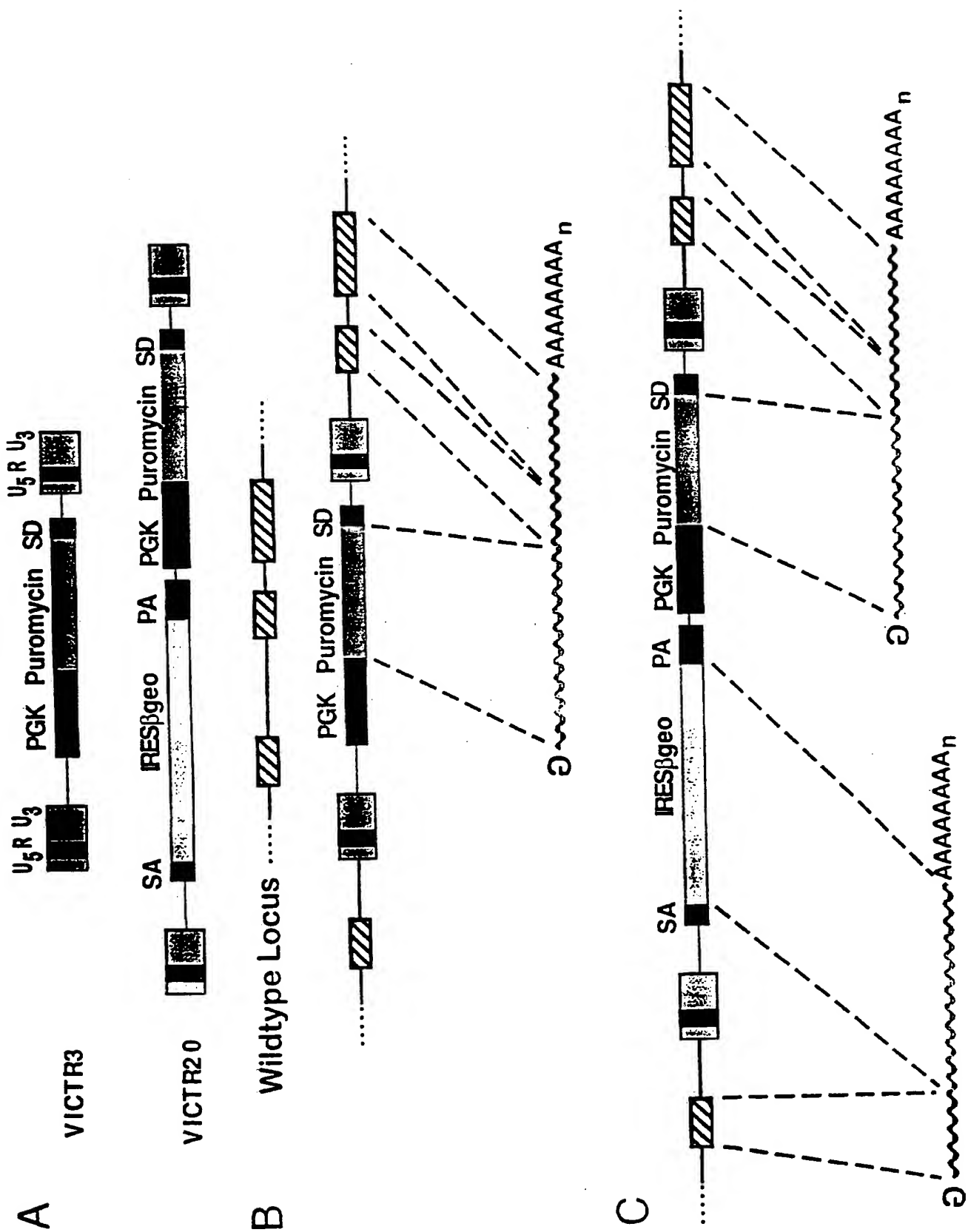


Figure 7

Figure 8

The following table includes 306 OSTs. OSTs with high into prodrom and Genbank patented sequences have been removed as well as sequence with repetitive elements hits.

[illegible]

Figure 8 cont'd.

05T281	gb U063113	1.8e-180	981	Mus musculus Mus musculus ras-GTPase-activating SH3-domain binding protein (GAP) gene, complete cDNA
05T295	gb AA048190	4.2e-60	811	Mus musculus m32911.11 Soaric mouse embryo NMEL13.5 14.5 Mus musculus cDNA clone 477500 5' similar to gb J02809
05T297	gb J17585	1.0e-168	101	Mouse neural specific calmodulin-binding protein P 57 mRNA, Mus musculus C57BL/6J (Hussum)
05T300	gb J75122	1.8e-203	981	Mus musculus House acid beta- galactosidase (GAL-1) gene, exon 16
05T301	gb J4850	2.7e-97	971	Mus musculus m62802.11 Soaric mouse embryo NMEL13.5 14.5 Mus musculus cDNA clone 35067 5' similar to gb U11211
05T311	gb U0422	3.0e-71	851	Mus musculus C57BL/6J (Hussum) protein 528 mRNA, complete Homo sapiens m88006.01 Soaric fetal heart NMH19M Homo sapiens cDNA clone J87147 3' similar to PIR:A51766 A51766
05T314	gb J14710	4.0e-54	731	metastasis-associated protein ma-1 cDNA 5' end similar to Hm
05T316	gb J1499	1.2e-72	991	Mus musculus m88002.11 Soaric mouse p19MF19.5 Mus musculus cDNA clone 317043 5' similar to SH:UCR5_00011
05T328	gb J10861	3.7e-59	891	P00130 UBQUINOL-CYTOSOLIC C REDUCTASE 7.2 KD PROTEIN Mus musculus m55503.11 Soaric mouse p19MF19.5 Mus musculus cDNA clone 314596 5'
05T331	gb J17698	6.8e-119	831	Mus musculus Mus musculus albitin-1 lacti-11 mRNA, complete cDNA
05T342	gb J10120	3.1e-143	951	Mus musculus Mus musculus 2002 mRNA, complete cDNA
05T356	gb J60456	1.8e-117	921	Mus musculus House cyclophilin mRNA, complete cDNA
05T361	gb J77360	5.7e-37	901	Mus musculus m65611.11 Soaric mouse embryo NMEL13.5 14.5 Mus musculus cDNA clone 40062 5'
05T368	gb J10662	2.7e-104	971	mus musculus House mouse, fibroblast domesticus mRNA for 14-3-3 (Lm), complete cDNA
05T386	gb J29946	2.6e-35	851	Mus musculus Mus musculus 94M genomic sequence encoding Tax gene
05T389	gb J15127	1.0e-70	891	Homo sapiens p12811.11 Homo sapiens cDNA clone 72500 5'
05T401	gb J09270	3.1e-31	971	Mus musculus m61901.11 Soaric mouse p19MF19.5 Mus musculus cDNA clone 340998 5' similar to SW:YFA1C01
05T411	gb J48542	2.0e-68	781	P12054 GDP-D-MANNOSE DEHYDRATASE Homo sapiens Y74907.01 Homo sapiens cDNA clone 276877 5'
05T418	gb J21163	1.7e-84	851	Homo sapiens human STS NF-15024 Homo sapiens House mRNA for
05T421	gb J25365	6.1e-56	861	Mus musculus House mRNA for preinvasive-like growth factor 1A
05T425	gb J04480	8.1e-58	991	Mus musculus m67907.11 Soaric mouse embryo NMEL13.5 14.5 Mus musculus cDNA clone 401244 5' similar to gb J23419
05T430	gb J07937	5.7e-93	961	INITIATION FACTOR 5A (HUMAN) Mus musculus House malle enzyme mRNA, complete cDNA
05T439	gb J0756	2.4e-134	871	Homo sapiens 1508 human ratino cDNA randomly primed sublibrary Homo
05T442	gb J02938	2.6e-49	701	sapiens cDNA H. sapiens mRNA for P1A12a protein
05T448	gb J07569	4.1e-72	881	Homo sapiens H. sapiens mRNA for C10 protein
05T511	gb J07591	3.1e-206	911	Mus musculus m50906.11 Soaric mouse embryo NMEL13.5 14.5 Mus musculus cDNA clone 39921 5' similar to gb J09659
05T536	gb J075415	4.6e-75	951	Mus musculus chaperonin 10 mRNA, complete cDNA (HOUSE)
05T547	gb J041004	4.1e-131	971	Mus musculus m61300.11 Soaric mouse p19MF19.5 Mus musculus cDNA clone 31156 5' similar to SW:K04_X1AC2 P35842 HYPOPHYSICAL 27.5 KD PROTEIN 10
05T562	gb J61433	7.6e-68	971	HOXD-10M1 INTERGENIC REGION, 111 Mus musculus M musculus mRNA for sodium/potassium ATPase beta subunit
05T564	gb J0007930	1.5e-11	671	Mus musculus m54007.11 Soaric mouse embryo NMEL13.5 14.5 Mus musculus cDNA clone 437748 5'
05T571	gb J0611270	2.1e-147	921	Mus musculus m63102.11 Life Tech mouse embryo 10 Sdpc 557307.5 Mus musculus cDNA clone 557307 5'
05T572	gb J0611047	1.2e-103	851	Homo sapiens z005910.11 Stratagene endothelial cell 917223 Homo sapiens cDNA clone 568850 3'
05T573	gb J06255	4.0e-69	751	Rattus norvegicus Rattus norvegicus RNA polymerase II transcription factor S111 p18 subunit mRNA, complete cda
05T577	gb J0020459	2.1e-91	921	Mus musculus m61606.11 Soaric mouse placenta 4NMP13.5 14.5 Mus musculus cDNA clone 435410 5'
05T581	gb J06552	2.0e-70	801	Homo sapiens Y05402.11 Homo sapiens cDNA clone 199610 5'
05T582	gb J07695	1.9e-218	911	Rattus rattus Rat mRNA for water channel aquaporin 3 (AQP3), complete cDNA
05T591	gb J061326	3.6e-103	851	Mus musculus Mus musculus domestica coiled-coil protein (CC-1) mRNA, complete cDNA
05T593	gb J070777	3.4e-117	981	Mus musculus m61402.11 Soaric mouse embryo NMEL13.5 14.5 Mus musculus cDNA clone 390314 5'
05T594	gb J064616	2.6e-142	871	Mus musculus M musculus mRNA for glycogen synthase
05T595	gb J067137	7.0e-51	861	Rattus norvegicus Rattus norvegicus PSD-95/SAP90-associated protein-1 mRNA, complete cDNA
05T598	gb J063476	2.2e-235	981	Mus musculus House mRNA for non-histone chromosomal protein HMG-14
05T600	gb J070494	1.0e-188	961	Mus musculus Mus musculus histone H2A.2 (H2A.2) mRNA, complete cda
05T607	gb J065702	1.2e-71	851	Mus musculus m61503.11 Life Tech mouse brain Mus musculus cDNA clone 312677 5'
05T611	gb J0610409	9.0e-68	971	Mus musculus m61505.11 Soaric mouse lymph nodes NMH19M Mus musculus cDNA clone 642392 5' similar to gb J00991
05T618	gb J011817	1.5e-95	861	Mus musculus m61107.11 Homo sapiens cDNA clone 47597 5'
05T620	gb J0617201	1.0e-78	831	Homo sapiens m625310.11 Heidelberg Mus musculus m625310.11 Heidelberg mouse embryonic testis Mus musculus cDNA clone 510918 5' similar to
05T623	gb J0600720	5.1e-106	811	gb J3559 Mus musculus Y-box binding protein mRNA, 3' end (HOUSE)
05T626	gb J061768	1.4e-47	911	Homo sapiens x183b02.11 Soaric fetal liver spleen INHLS 51 Homo sapiens cDNA clone 479851 5'
05T661	gb J06028410	1.2e-113	881	Homo sapiens human clone 192 Hsp-8 mRNA, partial cda
05T664	gb J011027	2.6e-106	871	Mus musculus m19406.11 Soaric mouse p19MF19.5 Mus musculus cDNA clone 463954 5' similar to gb J010775 House tau microtubule binding protein mRNA, complete (HOUSE)
05T671	gb J064860	8.1e-111	951	Mus musculus Mus musculus C57BL/6J Sec61 protein complex gamma subunit mRNA, complete cda
05T679	gb J061516	9.1e-119	971	Mus sp. Non/non-HOU domain-containing octamer-binding protein (mbc, U-cell leukemia, BCL1, MINA, 2411 nt)
05T680	gb J062558	4.2e-212	951	p19MF19.5 Mus musculus cDNA clone 330385 5'
05T702	gb J078993	5.7e-52	851	Mus musculus m61901.11 Soaric mouse p19MF19.5 Mus musculus cDNA clone 330385 5'
05T707	gb J0119122	1.2e-85	821	Mus musculus House stathmin gene Homo sapiens EST01041 Homo sapiens cDNA clone HMCPR34 similar to
05T716	gb J062791	4.1e-74	961	CAMP-regulated phosphoprotein Homo sapiens y6401.11 Homo sapiens cDNA clone 51271 5'
				Mus musculus m61609.11 Soaric mouse embryo NMEL13.5 14.5 Mus musculus cDNA clone 375104 5'

BNSDOCID: <WO__9814614A1_|_>

OST1096	gb 0870777	7.7e-112	861	(Cx31.1) mHNA, complete cds Homo sapiens human mHNA for KIA0233 gene, partial cds
OST1105	gb 0444231	1.0e-66	961	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1116	gb 0632249	3.1e-36	891	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1127	gb 0634485	1.2e-89	901	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1145	gb 080040	3.4e-128	911	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1152	gb 0679970	6.4e-109	101	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1156	gb 0101124	2.3e-65	951	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1165	gb 0634249	1.9e-184	971	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1179	gb AA008986	8.5e-84	941	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1186	gb 0899840	2.8e-70	961	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1192	gb 082490	5.3e-127	961	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1207	gb AA063763	1.3e-56	861	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1223	gb AA002931	1.5e-189	991	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1226	gb 037353	7.5e-279	951	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1233	gb 0498703	6.3e-91	981	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1234	gb 0895904	1.1e-180	981	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1241	gb 089850	4.8e-184	921	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1247	gb AA051266	4.7e-126	971	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1265	gb 0836073	6.8e-183	961	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1267	gb 084271	1.6e-32	861	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1269	gb 019977	4.0e-110	841	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1274	gb 0814634	2.1e-119	851	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1294	gb 0302803	6.8e-69	951	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1339	gb 0897190	3.1e-118	831	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1441	gb 0802611	7.8e-142	931	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1454	gb 0744481	2.2e-64	851	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1479	gb 0825672	5.1e-35	911	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1463	gb 082550	1.1e-109	971	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1376	gb 0836165	3.1e-33	921	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1397	gb 0335221	6.7e-27	931	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1410	gb 0802607	4.8e-17	891	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1429	gb 0802623	1.2e-122	971	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1447	gb 0802609	1.7e-145	941	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1453	gb 0338312	5.6e-92	861	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1457	gb 0878851	5.9e-45	801	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1470	gb 0802604	1.5e-136	951	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1478	gb 0878821	8.4e-55	801	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1488	gb 0802608	4.3e-235	941	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1492	gb 0802603	3.6e-109	981	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1493	gb AA0097148	3.5e-197	981	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1499	gb 0815184	1.3e-63	941	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1504	gb 0324260	1.7e-196	911	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1508	gb 0330306	1.8e-164	941	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1520	gb 0818420	4.7e-37	881	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1523	gb 0237069	7.2e-195	921	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1524	gb 085250	2.3e-168	961	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1526	gb AA117514	9.7e-120	891	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1528	gb 033765	2.2e-67	861	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1567	gb 0141636	1.7e-39	941	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1601	gb 0819611	1.3e-89	851	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1603	gb 0802922	4.0e-109	861	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1628	gb 08195129	3.9e-70	911	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1629	gb 08029090	7.9e-128	961	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1653	gb AA010795	5.1e-143	971	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1658	gb 0812941	5.2e-95	971	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1376	gb 0836165	3.1e-33	921	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1397	gb 0335221	6.7e-27	931	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1410	gb 0802607	4.8e-17	891	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1429	gb 0802623	1.2e-122	971	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1447	gb 0802609	1.7e-145	941	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1453	gb 0338312	5.6e-92	861	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1457	gb 0878851	5.9e-45	801	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1470	gb 0802604	1.5e-136	951	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1478	gb 0878821	8.4e-55	801	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1488	gb 0802608	4.3e-235	941	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1492	gb 0802603	3.6e-109	981	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1493	gb AA0097148	3.5e-197	981	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1499	gb 0815184	1.3e-63	941	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1504	gb 0324260	1.7e-196	911	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1508	gb 0330306	1.8e-164	941	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1520	gb 0818420	4.7e-37	881	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1523	gb 0237069	7.2e-195	921	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1524	gb 085250	2.3e-168	961	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1526	gb AA117514	9.7e-120	891	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1528	gb 033765	2.2e-67	861	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1567	gb 0141636	1.7e-39	941	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1601	gb 0819611	1.3e-89	851	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1603	gb 0802922	4.0e-109	861	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1628	gb 08195129	3.9e-70	911	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1629	gb 08029090	7.9e-128	961	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1653	gb AA010795	5.1e-143	971	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1
OST1658	gb 0812941	5.2e-95	971	Homo sapiens uc28804.01 Soares mouse serine/threonine kinase HsK1 serine/threonine kinase HsK1

OST2110	gb AA01277	6.5e-118	974	Mus musculus m14h08.r1 Soares mouse embryo NBHE13.5 14.5 Mus musculus cDNA clone 478911.5, similar to gb Y00764 UNIUQUINOL-CYTTOCHROME C REDUCTASE 11 NO PROTEIN (HUMAN)
OST2112	gb W05170	4.7e-118	964	Mus musculus m13101.r1 Soares mouse embryo NBHE13.5 14.5 Mus musculus cDNA clone 407817.5, similar to gb X00358 TUBULIN ALPHA-1 CHAIN (HUMAN); gb M13441 Mouse alpha-tubulin in 120yve M-alpha-6 mAb, complete cds
OST2116	gb X76453	2.4e-66	864	Matrix metalloproteinase-10 (MMP10) (HUMAN)
OST2136	gb W09817	1.8e-35	854	Mus musculus m13102.r1 Soares mouse embryo NBHE13.5 14.5 Mus musculus cDNA clone 35259.5
OST2134	gb W14001	5.2e-109	914	Mus musculus m64001.r1 Soares mouse embryo NBHE13.5 Mus musculus cDNA clone p19NF19.5, similar to p19NF250062.5 (NM_006613.5)
OST2141	gb J01351	3.8e-40	794	4E-BP1 protein - human
OST2165	gb W64236	1.7e-144	914	Homo sapiens yb0707.r1 Homo sapiens cDNA clone 240780.5
OST2174	gb W05055	1.1e-67	934	Mus musculus Mus musculus rpl1/rep2 interacting protein mRNA, complete cds
OST2177	gb W09250	1.9e-122	864	Mus musculus m160040.r1 Soares mouse embryo NBHE13.5 14.5 Mus musculus cDNA clone 418713.5
OST2182	gb W10048	2.3e-86	974	Mus musculus m67007.r1 Soares mouse p19NF19.5 Mus musculus cDNA clone 315733.5, similar to gb L03547 CLEAVAGE STIMULATION FACTOR, 50 KD SUBUNIT (HUMAN)
OST2191	gb U046834	5.4e-61	954	Mus musculus Mus musculus Shc mRNA, complete cds
OST2198	gb T25302	3.1e-33	694	Homo sapiens E57039R Homo sapiens cDNA clone BC19339R
OST2218	gb W01836	4.1e-112	974	Homo sapiens tcl1a1.s1 Soares parathyroid tumor NBH10A Homo sapiens cDNA clone J22288.3
OST2220	gb AAC19140	6.0e-153	934	Mus musculus m149C04.r1 Soares mouse embryo NBHE13.5 14.5 Mus musculus cDNA clone 479430.5, similar to SM-510-TORCA p1701 STRA1300REV11
OST2229	gb AA014563	2.8e-109	944	Mus musculus m167605.r1 Soares mouse embryo NBHE13.5 14.5 Mus musculus cDNA clone 468584.5
OST2236	gb W67358	2.4e-94	964	Mus sp. metallothionein-1 gene transcription activator (mice, L cells, mRNA, 509 nt) r1 Soares mouse
OST2237	gb AA002285	1.7e-33	104	Mus musculus m14115 Mus musculus cDNA embryo NBHE13.5, similar to cDNA clone 476848.5, similar to SK-1081-1EASR p18126 HYPOPHYSICAL 14.3 NO PROTEIN IN MUD1-AUD1 INTERGENIC REGION 11
OST2250	gb W71063	2.0e-137	964	Mus musculus m11402.r1 Soares mouse embryo NBHE13.5 14.5 Mus musculus cDNA clone 389114.5
OST2269	gb U046027	3.1e-119	944	Mus musculus Mus musculus CREB transcription factor, novel spliced isoform, partial cdy
OST2271	gb J031005	9.7e-111	944	Mus musculus Mus musculus tlc1.1 mRNA, complete cds
OST2272	gb W01026	1.7e-113	954	Mus musculus m12104.r1 Soares mouse p19NF19.5 Mus musculus cDNA clone 349591.5, similar to SM C1C1-D0V11
OST2283	gb W13930	1.2e-113	924	P15526 CILLOMINE CHANNEL PROTEIN PG4. Mus musculus m15408.r1 Soares mouse p19NF19.5 Mus musculus cDNA clone 332230.5, similar to gb U1115 LACTOYLGLUTATHIONE LYASE (HUMAN)
OST2296	gb W02037	8.3e-85	904	Mus musculus m169441.r1 Soares mouse embryo NBHE13.5 14.5 Mus musculus cDNA clone 403221.5, similar to

SH-VA06	SCHPO 009790	HYPOETHICAL 23 7
KL PROTEIN C116.14	IN CHROMOSOME 1	
KL sapiens il6. sapiens partial cDNA		
sequence: clone c-22607		
Mus musculus Mouse b52	MIHA lot 4	
novel protein		
kloma sapiens p19104	rl 1849gene	
colon (1837204)	kloma sapiens cDNA	
clone 512023 5		
nactus sp. Rat myoadenylate deaminase		
(AMP deaminase) mRNA, complete cds		
Mus musculus Mouse MIHA for		
synaptotagmin 1. Complete cds		
Mus musculus Mouse MIHA for Nip1p1122.		
complete cds		
kloma sapiens yd00492	rl kloma sapiens	
cDNA clone 21692 5		
Mus musculus mcl21009	rl Soares mouse	
p18MF19.5	Mus musculus cDNA clone	
249316		
Mus musculus mcl0404	rl Soares mouse	
p18MF19.5	Mus musculus cDNA clone	
317927 5		
Rattus norvegicus R norvegicus mRNA		
for carnitine/acetylarnitine carrier		
protein		
kloma sapiens m242406	rl Soares mouse	
placenta 418P13.5	14.5 Mus musculus	
cDNA clone 443434 5		
kloma sapiens kloma sapiens splicing		
factor (CC1) mRNA, complete cds		
Mus musculus mcl58110	rl Soares mouse	
embryo 018E13.5	14.5 Mus musculus cDNA	
clone 418483 5		
Mus musculus m198h06	rl Soares mouse	
p18MF19.5	Mus musculus cDNA clone	
484187 5		
Mus musculus m994912	rl Soares mouse	
embryo 018E13.5	14.5 Mus musculus cDNA	
clone 440710 5	similar to gb:M1690	
SH-VA06 SCHPO 010106	HYPOETHICAL 29.7	
KL PROTEIN C116.06	IN CHROMOSOME 1	
Mus musculus m33808	rl Soares mouse	
p18MF19.5	Mus musculus cDNA clone	
311671 5		
kloma sapiens Y422102	rl kloma sapiens	
cDNA clone 262491 5		
Mus musculus m05600	rl Life Tech	
mouse embryo 8 Sdpc 10646019	Mus	
musculus cDNA clone 557592 5	similar	
to gb:M1642	HYPOXANTHINE-GUANINE	
PHOSPHORIBOSYLTRANSFERASE (HGPRT);		
gb:J00423	mouse hypoxanthine	
phosphoribosyltransferase (hprt)mRNA		
Mus musculus mcl1904	rl Soares mouse	
p18MF19.5	Mus musculus cDNA clone	
348534 5		
Mus musculus m33007	rl Soares mouse	
p18MF19.5	Mus musculus cDNA clone	
483349 5	similar to M1-C12A3.]	
CED5343	COILED COIL DOMAINS	
kloma sapiens lq1806	rl 1849gene	
neuron (1937231)	kloma sapiens cDNA	
clone 631978 5		
Mus musculus Mus musculus		
co-chaperonin-1 collection A		
complete cds		
Mus musculus m14509	rl Soares mouse	
complete cds		
1.8e-116	941	
gb J02811	924	
gb AH060893	941	
gb H17623	854	
gb H100977	854	
gb H100666	991	
gb H18813	954	
gb H187811	854	
gb H187811	854	
gb AA011837	774	
gb L10911	914	
gb H87091	904	
gb AA080090	964	
gb AA015380	924	
gb U85614	914	
gb AA122609	104	
gb H14459	864	
gb AA049839	914	
gb H14179	944	
gb H125844	914	
gb AA104743	914	
gb H12442	914	
gb AA061143	944	
gb AA167801	904	
gb U051333	964	
gb H85263	944	

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Figure 8 cont'd.

OST2829	gb AA02649	7.7e-90	941	15S-binding protein embryo NMH13.5 14.5 Mus musculus cDNA clone 426106 5' similar to SW:MS5_HUMAN 000387 SEMIN PROTEIN MS55. 111 Mus musculus Mus musculus (1-terminal region) mRNA, complete cds
OST2834	gb U07632	1.4e-222	914	Mus musculus m79007.11 Soares mouse cDNA clone 540788 5' similar to gb:X83590 H. musculus mRNA (ol region) mRNA, complete cds
OST2835	gb AA060795	2.1e-89	914	Mus musculus m79007.11 Soares mouse cDNA clone 540788 5' similar to gb:X83590 H. musculus mRNA (ol region) mRNA, complete cds
OST2839	gb AA163971	6.0e-61	704	Mus musculus m79007.11 Soares mouse cDNA clone 540788 5' similar to gb:X83590 H. musculus mRNA (ol region) mRNA, complete cds
OST2842	gb U04515	6.1e-64	914	Mus musculus m79007.11 Soares mouse cDNA clone 540788 5' similar to gb:X83590 H. musculus mRNA (ol region) mRNA, complete cds
OST2877	gb J03583	1.3e-66	934	ADP-RIBOSYLATION FACTOR-LIKE PROTEIN 3 chain mRNA, complete cds
OST2883	gb U04850	4.0e-75	914	Mus musculus m79007.11 Soares mouse cDNA clone 540788 5' similar to gb:X83590 H. musculus mRNA (ol region) mRNA, complete cds
OST2892	gb U07798	1.4e-125	984	Mus musculus m79007.11 Soares mouse cDNA clone 540788 5' similar to gb:X83590 H. musculus mRNA (ol region) mRNA, complete cds
OST2897	gb U01107	7.9e-132	914	Mus musculus m79007.11 Soares mouse cDNA clone 540788 5' similar to gb:X83590 H. musculus mRNA (ol region) mRNA, complete cds
OST2909	gb AA166258	8.9e-120	964	Mus musculus m79007.11 Soares mouse cDNA clone 540788 5' similar to gb:X83590 H. musculus mRNA (ol region) mRNA, complete cds
OST2911	gb U073478	1.4e-117	864	Mus musculus m79007.11 Soares mouse cDNA clone 540788 5' similar to gb:X83590 H. musculus mRNA (ol region) mRNA, complete cds
OST2914	gb U02236	4.0e-136	954	Mus musculus m79007.11 Soares mouse cDNA clone 540788 5' similar to gb:X83590 H. musculus mRNA (ol region) mRNA, complete cds
OST2916	gb U077002	1.4e-67	924	Mus musculus m79007.11 Soares mouse cDNA clone 540788 5' similar to gb:X83590 H. musculus mRNA (ol region) mRNA, complete cds
OST2921	gb U075740	8.4e-106	984	Mus musculus m79007.11 Soares mouse cDNA clone 540788 5' similar to gb:X83590 H. musculus mRNA (ol region) mRNA, complete cds
OST2922	gb U050544	8.4e-135	884	Mus musculus m79007.11 Soares mouse cDNA clone 540788 5' similar to gb:X83590 H. musculus mRNA (ol region) mRNA, complete cds
OST2923	gb U05631	3.2e-108	974	Mus musculus m79007.11 Soares mouse cDNA clone 540788 5' similar to gb:X83590 H. musculus mRNA (ol region) mRNA, complete cds
OST2926	gb U059561	6.1e-164	944	Mus musculus m79007.11 Soares mouse cDNA clone 540788 5' similar to gb:X83590 H. musculus mRNA (ol region) mRNA, complete cds
OST2929	gb U05735	3.0e-92	924	Mus musculus m79007.11 Soares mouse cDNA clone 540788 5' similar to gb:X83590 H. musculus mRNA (ol region) mRNA, complete cds
OST2934	gb U042904	1.8e-75	934	Mus musculus m79007.11 Soares mouse cDNA clone 540788 5' similar to gb:X83590 H. musculus mRNA (ol region) mRNA, complete cds
OST2940	gb AA154035	1.4e-114	974	Mus musculus m79007.11 Soares mouse cDNA clone 540788 5' similar to gb:X83590 H. musculus mRNA (ol region) mRNA, complete cds
OST2942	gb U04882	1.4e-91	964	Mus musculus m79007.11 Soares mouse cDNA clone 540788 5' similar to gb:X83590 H. musculus mRNA (ol region) mRNA, complete cds
OST2948	gb AA108292	5.1e-32	814	Rattus norvegicus EST0035 rat 14kDa ZAP1 library (cDNA clone 1409) 5' similar to ADP-ribosylation factor (ARF1)-like protein
OST2953	gb U010606	1.8e-97	984	Mus musculus m79007.11 Soares mouse cDNA clone 540788 5' similar to gb:X83590 H. musculus mRNA (ol region) mRNA, complete cds
OST2956	gb AA049172	1.1e-147	974	Mus musculus m79007.11 Soares mouse cDNA clone 540788 5' similar to gb:X83590 H. musculus mRNA (ol region) mRNA, complete cds
OST2963	gb U004744	4.2e-31	804	embryo NMH13.5 14.5 Mus musculus cDNA clone 479149 5' similar to MP:145812.4 C02740 Homo sapiens 1379c08.11 Soares (etal) 1309 NMH13.5 Mus sapiens cDNA clone 289766 5' Mus musculus m72607.11 Beakington cDNA clone 537733 5' similar to SM:YH51 YEAST Y38219 HYPOTHETICAL 44.2 KD PROTEIN IN SC02-MHF1 INTERGENIC REGION
OST2971	gb AA120487	9.2e-107	104	Rattus norvegicus Rattus norvegicus neuroglycan C precursor mRNA, complete cds
OST2973	gb U033553	2.6e-192	884	Mus musculus m79007.11 Soares mouse cDNA clone 540788 5' similar to gb:X83590 H. musculus mRNA (ol region) mRNA, complete cds
OST2977	gb U07755	6.1e-164	974	Mus musculus m79007.11 Soares mouse cDNA clone 540788 5' similar to gb:X83590 H. musculus mRNA (ol region) mRNA, complete cds
OST2981	gb AA206420	1.2e-71	854	Homo sapiens XQ1002.21 Stratagene neuroepithelium (1937231) Homo sapiens cDNA clone 645099 3' similar to TR:Q972006 Q972006 mRNA: EXPRESSED SEQUENCE TAG
OST2983	gb U049206	1.1e-119	904	Mus musculus m79007.11 Soares mouse cDNA clone 540788 5' similar to gb:X83590 H. musculus mRNA (ol region) mRNA, complete cds
OST2987	gb AA029603	2.3e-134	904	Mus musculus m79007.11 Soares mouse cDNA clone 540788 5' similar to gb:X83590 H. musculus mRNA (ol region) mRNA, complete cds
OST2988	gb X52129	2.2e-52	734	Mus musculus m79007.11 Soares mouse cDNA clone 540788 5' similar to gb:X83590 H. musculus mRNA (ol region) mRNA, complete cds
OST2989	gb AA152050	1.3e-46	784	Mus musculus m79007.11 Soares mouse cDNA clone 540788 5' similar to gb:X83590 H. musculus mRNA (ol region) mRNA, complete cds
OST2991	gb AA003171	8.4e-151	934	Mus musculus m79007.11 Soares mouse cDNA clone 540788 5' similar to gb:X83590 H. musculus mRNA (ol region) mRNA, complete cds
OST2994	gb U051546	1.9e-51	814	Mus musculus m79007.11 Soares mouse cDNA clone 540788 5' similar to gb:X83590 H. musculus mRNA (ol region) mRNA, complete cds
OST2996	gb X99921	1.6e-82	104	Mus musculus m79007.11 Soares mouse cDNA clone 540788 5' similar to gb:X83590 H. musculus mRNA (ol region) mRNA, complete cds
OST2998	gb U019012	3.2e-48	104	Mus musculus m79007.11 Soares mouse cDNA clone 540788 5' similar to gb:X83590 H. musculus mRNA (ol region) mRNA, complete cds
OST3003	gb U07502	3.3e-169	974	Mus musculus m79007.11 Soares mouse cDNA clone 540788 5' similar to gb:X83590 H. musculus mRNA (ol region) mRNA, complete cds
OST3004	gb AA103385	1.9e-162	984	Mus musculus m79007.11 Soares mouse cDNA clone 540788 5' similar to gb:X83590 H. musculus mRNA (ol region) mRNA, complete cds
OST3011	gb AA035005	1.2e-98	994	Mus musculus m79007.11 Soares mouse cDNA clone 540788 5' similar to gb:X83590 H. musculus mRNA (ol region) mRNA, complete cds
OST3017	gb U050908	4.8e-123	924	Mus musculus m79007.11 Soares mouse cDNA clone 540788 5' similar to gb:X83590 H. musculus mRNA (ol region) mRNA, complete cds
OST3018	gb U083277	2.2e-235	994	Mus musculus m79007.11 Soares mouse cDNA clone 540788 5' similar to gb:X83590 H. musculus mRNA (ol region) mRNA, complete cds
OST3032	gb U049165	2.1e-76	994	Mus musculus m79007.11 Soares mouse cDNA clone 540788 5' similar to gb:X83590 H. musculus mRNA (ol region) mRNA, complete cds
OST3035	gb U08051	1.8e-115	904	Mus musculus m79007.11 Soares mouse cDNA clone 540788 5' similar to gb:X83590 H. musculus mRNA (ol region) mRNA, complete cds
OST3037	gb U090766	4.5e-34	744	Mus musculus m79007.11 Soares mouse cDNA clone 540788 5' similar to gb:X83590 H. musculus mRNA (ol region) mRNA, complete cds

OST33105	gb U08153	1.0e-106	874	Mus musculus Mus musculus
OST33112	gb U08109	9.7e-59	664	prepro-neurexin mRNA, complete cds
OST33121	gb U041643	1.2e-112	914	Mus musculus Mouse YL-1 mRNA for YL-1 protein (involves protein with DNA-binding ability), complete cds
OST33124	gb U041199	2.2e-51	874	Mus musculus Mouse 152 mRNA for a novel protein
OST33125	gb U028476	6.5e-103	944	homo sapiens human mRNA for KIAA0045 gene, complete cds
OST33149	gb U0418210	2.2e-52	944	Mus musculus Mouse P51-3 factor 5-11, clone P51-3
OST33152	gb AA009550	4.9e-63	774	homo sapiens HAP100 Homo sapiens cDNA clone 407679 J
OST33154	gb U023670	9.1e-69	924	Mus musculus mdrl401 r1 Soares mouse embryo NMEL13.5 14.5 Mus musculus cDNA clone 368731.5 14.5 Mus musculus SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11)
OST33155	gb U0491805	4.1e-40	824	Mus musculus mdrl401 r1 Soares mouse embryo NMEL13.5 14.5 Mus musculus SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11)
OST33166	gb AA122835	2.1e-85	694	Mus musculus mdrl401 r1 Soares mouse embryo NMEL13.5 14.5 Mus musculus SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11)
OST33170	gb U050758	4.6e-106	944	Mus musculus mdrl401 r1 Soares mouse embryo NMEL13.5 14.5 Mus musculus SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11)
OST33171	gb U031107	1.5e-50	714	Mus musculus mdrl401 r1 Soares mouse embryo NMEL13.5 14.5 Mus musculus SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11)
OST33172	gb U048459	2.2e-134	934	Mus musculus mdrl401 r1 Soares mouse embryo NMEL13.5 14.5 Mus musculus SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11)
OST33175	gb AA015237	4.0e-44	104	Mus musculus mdrl401 r1 Soares mouse embryo NMEL13.5 14.5 Mus musculus SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11)
OST33176	gb U027347	4.2e-103	994	Mus musculus mdrl401 r1 Soares mouse embryo NMEL13.5 14.5 Mus musculus SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11)
OST33188	gb U050264	1.9e-117	984	Mus musculus mdrl401 r1 Soares mouse embryo NMEL13.5 14.5 Mus musculus SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11)
OST33190	gb U041022	3.6e-46	784	Mus musculus mdrl401 r1 Soares mouse embryo NMEL13.5 14.5 Mus musculus SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11)
OST33193	gb U060310	1.7e-208	934	Mus musculus mdrl401 r1 Soares mouse embryo NMEL13.5 14.5 Mus musculus SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11)
OST33194	gb AA168895	6.3e-109	984	Mus musculus mdrl401 r1 Soares mouse embryo NMEL13.5 14.5 Mus musculus SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11)
OST33197	gb U021817	3.3e-35	914	Mus musculus mdrl401 r1 Soares mouse embryo NMEL13.5 14.5 Mus musculus SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11)
OST33195	gb U071116	1.3e-105	884	Mus musculus mdrl401 r1 Soares mouse embryo NMEL13.5 14.5 Mus musculus SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11)
OST33198	gb AA189139	3.4e-37	884	Mus musculus mdrl401 r1 Soares mouse embryo NMEL13.5 14.5 Mus musculus SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11)
OST33199	gb U051858	7.9e-66	774	Mus musculus mdrl401 r1 Soares mouse embryo NMEL13.5 14.5 Mus musculus SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11)
OST33200	gb U058426	7.1e-53	964	Mus musculus mdrl401 r1 Soares mouse embryo NMEL13.5 14.5 Mus musculus SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11)
OST33207	gb U047064	9.0e-166	974	Mus musculus mdrl401 r1 Soares mouse embryo NMEL13.5 14.5 Mus musculus SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11)
OST33210	gb AA189121	4.2e-114	924	Mus musculus mdrl401 r1 Soares mouse embryo NMEL13.5 14.5 Mus musculus SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11)
OST33280	gb AA118567	9.3e-100	894	Mus musculus mdrl401 r1 Soares mouse embryo NMEL13.5 14.5 Mus musculus SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11)
OST33281	gb U045606	1.0e-121	954	Mus musculus mdrl401 r1 Soares mouse embryo NMEL13.5 14.5 Mus musculus SH:NMK3_HUMAN 125540 NMK3 (MURF1) (11) SH:NMK3_HUMAN 125540 NMK3

Figure 8 cont'd.

05T3788	gb AA014126	9,7c-25	101	Mus musculus m64001.01 Soares mouse embryo NMHE13.5 14.5 Mus musculus cDNA clone 43657.5. similar to SM-NM7. NOV11 002167 NMH-OUTTHONE. OXIDOREDUCTASE 017 SUBUNIT
05T3789	gb U13544	9,5c-67	971	Mus musculus mouse mRNA for primase small subunit, complete cds
05T3807	gb U26968	3,8c-51	801	Human sapiens 1617 human retina cDNA randomly primed cDNA library Homo sapiens cDNA
05T3818	gb U26231	3,8c-48	961	Human sapiens 1617 human retina cDNA randomly primed cDNA library Homo sapiens cDNA
05T3819	gb U26232	3,8c-45	811	Human sapiens 1617 human retina cDNA randomly primed cDNA library Homo sapiens cDNA
05T3827	gb AA046400	1,2c-67	841	Human sapiens 1617 human retina cDNA randomly primed cDNA library Homo sapiens cDNA
05T3833	gb U70777	3,3c-121	991	Mus musculus m64002.01 Soares mouse embryo NMHE13.5 14.5 Mus musculus cDNA clone 390314.5
05T3839	gb U86008	1,4c-103	861	Human sapiens 1617 human retina cDNA randomly primed cDNA library Homo sapiens cDNA
05T3843	gb U282190	2,8c-51	881	Human sapiens 1617 human retina cDNA randomly primed cDNA library Homo sapiens cDNA
05T3849	gb U64986	1,3c-173	941	Mus musculus m64005.01 Soares mouse embryo NMHE13.5 14.5 Mus musculus cDNA clone 386504.5. similar to SM-USH7. DICD1. P14327 VEGTATIVE SPECIFIC PROTEIN 07. 111
05T3851	gb U51037	1,0c-135	841	Mus musculus m64006.01 Soares mouse embryo NMHE13.5 14.5 Mus musculus cDNA clone 351419.5. similar to SM-USH7. DICD1. P14327 VEGTATIVE SPECIFIC PROTEIN 07. 111
05T3858	gb X56135	4,7c-237	971	Mus musculus m64007.01 Soares mouse embryo NMHE13.5 14.5 Mus musculus cDNA clone 374619.5. similar to gb U07151
05T3864	gb U19493	9,8c-33	951	Mus musculus m64008.01 Soares mouse embryo NMHE13.5 14.5 Mus musculus cDNA clone 351419.5. similar to SM-USH7. DICD1. P14327 VEGTATIVE SPECIFIC PROTEIN 07. 111
05T3869	gb U41525	4,4c-100	851	Mus musculus m64009.01 Soares mouse embryo NMHE13.5 14.5 Mus musculus cDNA clone 351419.5. similar to SM-USH7. DICD1. P14327 VEGTATIVE SPECIFIC PROTEIN 07. 111
05T3877	gb U410485	3,8c-97	951	Mus musculus m64010.01 Soares mouse embryo NMHE13.5 14.5 Mus musculus cDNA clone 314334.5
05T3903	gb U659188	1,2c-118	861	Mus musculus m64011.01 Soares mouse embryo NMHE13.5 14.5 Mus musculus cDNA clone 374619.5. similar to gb U07151
05T3905	gb U095430	1,0c-102	921	Mus musculus m64012.01 Soares mouse embryo NMHE13.5 14.5 Mus musculus cDNA clone 374619.5. similar to gb U07151
05T3909	gb AA020459	1,2c-80	941	Mus musculus m64013.01 Soares mouse embryo NMHE13.5 14.5 Mus musculus cDNA clone 455810.5
05T3917	gb U243034	0,7c-81	871	Human sapiens 1617 human retina cDNA randomly primed cDNA library Homo sapiens cDNA
05T3924	gb U204099	3,9c-32	841	Human sapiens 1617 human retina cDNA randomly primed cDNA library Homo sapiens cDNA
05T3925	gb U235111	1,2c-88	761	Human sapiens 1617 human retina cDNA randomly primed cDNA library Homo sapiens cDNA
05T3931	gb U14957	1,6c-36	811	Human sapiens 1617 human retina cDNA randomly primed cDNA library Homo sapiens cDNA
05T3945	gb U101004	1,6c-152	971	Human sapiens 1617 human retina cDNA randomly primed cDNA library Homo sapiens cDNA
05T3957	gb AA051293	2,8c-143	961	Human sapiens 1617 human retina cDNA randomly primed cDNA library Homo sapiens cDNA
05T3960	gb U38614	1,1c-88	821	Human sapiens 1617 human retina cDNA randomly primed cDNA library Homo sapiens cDNA
05T3961	gb U657908	6,6c-37	771	Human sapiens 1617 human retina cDNA randomly primed cDNA library Homo sapiens cDNA

Figure 8 cont'd.

OST4196	gb W41301	3.1e-39	98%	Mus musculus mcl306.r1 Soares mouse p1MF19.5 Mus musculus cDNA clone 351223 5'.
OST4223	gb AA20378V	2.7e-89	90%	Mus musculus mcl012.r1 Soares mouse lymph node NIMIN Mus musculus cDNA clone 643823 5'.
OST4228	gb S51016	9.3e-205	92%	Bos taurus Z2125K1multibiquitinating enzyme [cattle, thymus, mRNA, 825 nt]
OST4229	gb Z31263	4.8e-70	97%	Mus musculus M.musculus expressed sequence tag MTEST7
OST4235	gb W53187	3.0e-173	97%	Mus musculus md1907.r1 Soares mouse embryo NBME13.5 14.5 Mus musculus cDNA clone 368820 5' similar to MP:C32D5.9 CD1849
OST4242	gb AA048921	2.3e-40	86%	Mus musculus mJ7611.r1 Soares mouse embryo NBME13.5 14.5 Mus musculus cDNA clone 479276 5' similar to gb:U11705
OST4245	gb H10216	9.9e-80	75%	Mus musculus domestica C57BL/6J plasma glutathione (HMOSE)
OST4247	gb AA023146	1.5e-115	96%	Homo sapiens ym02105.s1 Homo sapiens cDNA clone 46710 3'
OST4251	gb AA070774	8.7e-154	98%	Mus musculus mcl603.r1 Soares mouse placenta (HMP13.5 14.5 Mus musculus cDNA clone 455281 5' similar to SK:A47-MUNM 004941 INTSTINAL
OST4254	gb H54737	2.4e-82	10%	Homo sapiens ym02105.s1 Stratagene (HMOSE) (HMOSE) (HMOSE)
OST4258	gb AA013789	4.3e-169	90%	Mus musculus mcl0404.r1 Soares mouse placenta (HMP13.5 14.5 Mus musculus cDNA clone 442371 5' similar to PIR:JC2472 JC2472 RE protein - human
OST4281	gb U16175	4.0e-40	63%	Mus musculus Mus musculus thrombospondin 3 (Thbs3) gene, partial cds and eucin 1 (Nuc1) gene, complete cds
OST4283	gb AA007519	8.9e-52	81%	Homo sapiens th98e12.r1 Soares fetal liver spleen INFLS S1 Homo sapiens cDNA clone 429358 5'.
OST4288	gb AA000024	1.4e-135	96%	Mus musculus mcl306.r1 Soares mouse embryo NBME13.5 14.5 Mus musculus cDNA clone 425602 5' similar to gb:X03920_rna2 M.musculus GSNPX gene (HMOSE)
OST4315	gb H18210	6.4e-62	96%	Mus musculus Mouse transcription factor S-II, clone PS11-3
OST4319	gb J04696	2.0e-127	95%	Mus musculus Mouse glutathione S-transferase class mu (GSTS-5) mRNA, complete cds
OST3971	gb W45926	9.6e-55	94%	Mus musculus mcl306.r1 Soares mouse embryo NBME13.5 14.5 Mus musculus cDNA clone 354750 5'.
OST3988	gb H13524	2.6e-111	90%	Mus musculus Mus musculus amyloid A pseudogene (pml-SM)
OST3993	gb H16778	4.7e-45	82%	Homo sapiens ym02105.s1 Homo sapiens cDNA clone 128514.07.r1 Soares mouse embryo NBME13.5 14.5 Mus musculus cDNA clone 425106 5'.
OST4002	gb AA000314	1.9e-112	96%	Mus musculus mcl306.r1 Soares mouse embryo NBME13.5 14.5 Mus musculus cDNA clone 425106 5'.
OST4003	gb J37297	2.9e-121	91%	Mus musculus Mus musculus (clone 86) Myeloid 13us Mus musculus expressed sequence tag EST P032
OST4011	gb J26664	2.0e-155	94%	Homo sapiens human mRNA for KIAA0780 gene, partial cds
OST4028	gb J087470	7.5e-93	92%	Homo sapiens hmo5104.s1 Stratagene hnt neuron (H937233) Homo sapiens cDNA clone 545559 3' similar to TR:G600529
OST4033	gb AA084704	2.2e-54	88%	G600529 NADH UBIQUINONE OXIDOREDUCTASE SUBUNIT
OST4051	gb P03500	7.8e-63	86%	Homo sapiens H. sapiens partial cDNA sequence; clone c-1ld08
OST4061	gb W0618	3.1e-118	97%	Mus musculus mcl012.r1 Soares mouse p1MF19.5 Mus musculus cDNA clone 348167 5'.
OST4070	gb W35515	6.0e-135	94%	Mus musculus mcl603.r1 Soares mouse p1MF19.5 Mus musculus cDNA clone 335388 5'.
OST4073	gb X82021	2.0e-105	91%	Rattus norvegicus R. norvegicus mRNA for heat shock related protein
OST4074	gb J063704	3.1e-140	86%	Rattus norvegicus Rat mRNA for dihydroxyisovalinate, complete cds
OST4106	gb W75804	1.1e-84	93%	Mus musculus mcl603.r1 Soares mouse embryo NBME13.5 14.5 Mus musculus cDNA clone 400594 5'.
OST4114	gb W20730	6.5e-90	96%	Mus musculus mcl603.r1 Soares mouse p1MF19.5 Mus musculus cDNA clone 337286 5'.
OST4131	gb AA044274	2.4e-33	69%	Homo sapiens hmo5104.s1 Soares pregnant uterus NBMPU Homo sapiens cDNA clone 486677 3'.
OST4134	gb H131489	3.0e-84	85%	Rattus sp. EST105564 Rattus sp. cDNA 3' end
OST4140	gb W71052	3.7e-121	91%	Mus musculus mcl603.r1 Soares mouse embryo NBME13.5 14.5 Mus musculus cDNA clone 389725 5'.
OST4142	gb C07091	5.7e-74	88%	SW:18MCTEAT P18182 HYPOPHYSICAL 13.6 REGION. (1)
OST4144	gb X56135	4.4e-61	83%	Rattus norvegicus similar to none Rattus norvegicus alpha
OST4148	gb W54510	1.5e-134	91%	prothymosin alpha
OST4149	gb U36393	2.6e-111	96%	Mus musculus Mus musculus AS8059 protein-tyrosine-phosphatase transcription factor TFE8 mRNA, partial cds
OST4154	gb X56046	1.3e-161	96%	Mus musculus Mouse mRNA (clone lambda-181) for hypothetical protein A
OST4155	gb X05900	3.5e-58	85%	Rattus norvegicus Rat mRNA for lens beta2I-crystallin (PRLB2A 01-3)
OST4166	gb U53859	8.0e-169	90%	Rattus norvegicus Rattus norvegicus calpain small subunit (Cas1) mRNA, partial cds
OST4174	gb U41395	1.3e-38	84%	Mus musculus Mus musculus X inactive species transcript (Xiac) gene, partial cds
OST4191	gb H63507	2.0e-76	81%	muscid 18A-10A fragment 2
OST4192	gb W51557	2.2e-83	82%	Mus musculus M.musculus 10X-3.5 gene Mus musculus mcl603.r1 Soares mouse embryo NBME13.5 14.5 Mus musculus cDNA clone 408455 5' similar to SK:G1H-HUMAN P24897 SERINE PROTEIN KINASE TRANSFERASE, MITOCHONDRIAL
OST4194	gb W24615	8.9e-38	87%	Mus musculus mcl306.r1 Soares mouse p1MF19.5 Mus musculus cDNA clone 350148 5'.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/17791

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C12Q 1/68; C12N 5/02, 5/06, 15/00, 15/64; C07H 21/04

US CL : 435/6, 320.1, 325, 357; 536/23.1, 24.2; 800/2

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 320.1, 325, 357; 536/23.1, 24.2; 800/2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS and DIALOG

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SAUER, B. Site-specific recombination; developments and applications. Current Opinion in Biotechnology. May 1994, Vol. 5, pages 521-527, see the entire article.	1-8, 10, 20 and 28
Y	SEKINE et al. Frameshifting is required for production of the transposase encoded by insertion sequence 1. Proc. Natl. Acad. Sci. USA. June 1989, Vol. 86, pages 4609-4613, see especially "Frameshifting in Other Systems", page 4613.	10
X	WANG, et al. High frequency recombination between loxP sites in human chromosomes mediated by an adenovirus vector expressing Cre recombinase. Somatic Cell and Molecular Genetics. 09 March 1996, Vol. 21, No. 6, pages 429-441, see especially the abstract.	8

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

30 JANUARY 1998

Date of mailing of the international search report

02 MAR 1998

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/17791

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ODELL et al. Site-directed recombination in the genome of transgenic tobacco. Molecular and General Genetics. 11 October 1990, Vol. 223, pages 369-378, see especially Figure 1 and the "Result" section.	1-8, 10, 20
X	DYMECKI, S. A modular set of Flp, FRT and LacZ fusion vectors for manipulating genes by site-specific recombination. Gene. 01 June 1996, Vol. 171, pages 197-201, see especially Figure 1.	10
X	HAAS et al. TnMax - a versatile mini-transposon for the analysis of cloned genes and shuttle mutagenesis. Gene. 11 August 1993, Vol. 130, pages 23-31, see especially the abstract.	8
Y	WO 88/01646 (ALLELIX INC.) 10 March 1988 (10.10.88), see especially pages 1-3.	1-8, 10 and 20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/17791

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-8, 10, 20 and 28

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1.

Group I, claim(s) 1-7, 8, 10, 20 and 28, drawn to a library of cultured eucaryotic cells made by a process comprising treating a group of cells with a vector that mediates the splicing of a foreign exon internal to a cellular transcript, the use of the cell from the library to generate a non-human transgenic animal, and the method of making the cell comprising the vector and the use of the vector to make the library of cultured eukaryotic cells.

Group II, claim(s) 9, 11-18, drawn to a vector construct for replacing the 3' end of an animal cell transcript with a foreign exon.

Group III, claim(s) 19, 21 and 22, drawn to the use of a vector according to claim 9.

Group IV, claim 23, drawn to a stably transduced animal cell that incorporates the vector of claim 16.

Group V, claims 24-27, drawn to a method of altering a region of DNA by adding or deleting DNA.

The inventions listed as Groups I-V do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the first group contains the product, a library of cultured eukaryotic cell, a method of using the cells to produce a non-human transgenic animal and a method of making the cells. The additional groups are directed to different vectors having different compositions than the vector used in the first group, cell lines containing those vector constructs and methods of altering the cellular genome. The first group contains a vector having a different composition than the other vectors and therefore the special technical feature present in the first group does not occur in the other groups.

